1998

Prevention of Hydrolytic and Oxidative Rancidity and Nutrient Losses in Rice Bran During Storage.

Fatemeh Malekian Ramezanzadeh
Louisiana State University and Agricultural & Mechanical College

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

PREVENTION OF HYDROLYTIC AND OXIDATIVE RANCIDITY AND NUTRIENT LOSSES IN RICE BRAN DURING STORAGE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by
Fatemeh Malekian Ramezanzadeh
B.S., Tehran University, Tehran, Iran, 1972
M.S., Louisiana State University, 1992
December, 1998

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

ACKNOWLEDGEMENTS

I wish to express my gratitude and deepest appreciation to my major professors, Dr. Ramu M. Rao and Dr. Marlene Windhauser for their encouragement, guidance, constructive criticism, and assistance throughout the research and preparation of this dissertation. I am grateful to Dr. Joseph A. Liuzzo, Dr. Witoon Prinyawiwatkul, Dr. Douglas L. Park, Head of the Food Science Department, and Dr. Lalit R. Verma, Dean's Representative, for serving on my advisory committee and for their valuable advice.

The author sincerely thanks Dr. George Bray, Dr. Donna Ryan, and Dr. Richard Tulley for permitting this research in the Food Analysis Laboratory at Pennington Biomedical Research Center. Special appreciation is extended to Dr. Charls J. Monlezun, Department of Experimental Statistics, for valuable advice. I wish to gratefully acknowledge Dr. Ruth Patrick, Dr. Sun Yoo, Regina Louviere, Gail and John Bley, and Dianne Brikner for friendship, endless support, advice, and encouragement. I am very grateful to Claire Lassalle and Susan Clinkenbeard for their technical assistance and kind advice.

Finally, I wish to dedicate this work to my husband, Ghafoor Ramezanzadeh (Ramsey), my father, Davood Malekian, my mother, Zahra Olphat, my daughters, Elizeh, Elahe, Julie, and Crystal, and my granddaughter Sarah. Their encouragement, love, patience, and understanding have made this work possible. The author thanks God for being there at all times.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Freshly milled raw rice bran was stabilized with microwave heat for 3 min. Half of raw and treated bran were packed in Ziplock® bags and the other half vacuum packed. Half of the samples were stored at 4-5°C and the other half at 25°C for 16 weeks. The free fatty acid (FFA) content increased rapidly in raw rice bran samples vacuum packed and samples packed in Ziplock® bags store at 25°C from an initial amount of 2.5% to 54.3% and 48.1%, respectively. FFA level increased significantly in raw samples vacuum packed and packed in Ziplock® bags stored 4-5°C from an initial amount of 2.5% to 25.4% and 19.5%, respectively. Microwave heat stabilized samples stored at 25°C had a significant increase in FFA level from an initial amount of 2.8% to 11.6% and 10.9% respectively, for vacuum packed and samples stored in the Ziplock® bags. FFA in microwave heat stabilized samples stored 4-5°C did not increase during 16 weeks of storage. Rice bran lipoxygenase had optimum pH of 8.5 and optimum temperature of 25-30°C. Michaelis constant and maximum velocity were 0.097 mM and 0.847 dA/min, respectively. Lipoxygenase activity in microwave heat stabilized samples in Ziplock® bags or under vacuum and stored at 25°C showed a significant increase from an initial amount of 0.18 dA/min to 0.54 and 0.26 dA/min, respectively. Lipoxygenase activity for microwave heat stabilized samples packed in both packing methods did not change significantly from the initial amount of 0.18 dA/min to 0.15 dA/min and 0.14 dA/min when stored at 4-5°C at 12 weeks. A significant decrease to 0.08 dA/min and 0.07 dA/min was observed at week 16. Fatty

acid composition, especially linoleic acid, did not show any significant changes for the microwave heat stabilized samples stored at 4-5°C Proximate composition did not change except for moisture content which decreased significantly from an initial amount of 8.4% to 6.3%. No advantage was found to vacuum packing the samples. The combination of 4-5°C storage temperature and Ziplock® bags provided the best shelf-life for microwave heat stabilized rice bran during 16 weeks of storage, under the conditions employed in this study.

CHAPTER 1
INTRODUCTION

For more than one hundred years, the developed nations have used roller milling to separate bran from wheat grain to produce white flour. In the absence of bran the flour had a storage stability much superior to that of whole meal; bran was a byproduct, fed to animals, and little interest was shown in its stability. Since 1970, nutritionists have recommended a greater consumption of dietary fiber, and cereal bran began to play a much more promising role, both as a food ingredient and as a component of brown flour which deteriorates much faster than does white flour during storage. The alterioration is often attributed to the onset of rancidity in bran (Barnes and Galliard, 1991). Rancidity is due to changes in the lipid fraction of bran. The polyunsaturated fatty acids (PUFA) such as linoleic acid in rice bran are essential nutrients in our diet, but they can challenge the food scientist for further research.

Rapid oil deterioration of rice bran by lipase and, to a lesser extent, oxidase, immediately following the milling process makes the bran unfit for human food. However, if properly handled, rice bran can serve as a rich source of nutrients, with nearly 14-16% protein, 15-23% fat and 8-10% fiber. Three major fatty acids, palmitic acid, oleic acid (40-50%) and linoleic acid (30- 35%) make up about 90% of total fatty acids. Rice bran oil is similar in composition to sesame, corn, cottonseed, and peanut oils (Saunders, 1985). Rice bran is also a good source of B vitamins and contains minerals such as iron, potassium, calcium, magnesium, and manganese. Furthermore, the United States Department of Agriculture (USDA) findings show that rice bran is as

good as or better than oat bran in reducing serum cholesterol, increasing water retention in the feces, and reducing the risk of heart diseases (Urbanski, 1990).

Immediately after the completion of rice milling, the lipases naturally occurring in rice bran decompose triglycerols (TG) which are the primary lipids in rice bran. The resulting fatty acids become susceptible to oxidation; acidity increases, pH reduces, an off-flavor and soapy taste is produced, and functional properties change. Rice bran contains several types of lipases that are site-specific and cleave the 1,3-site of triglycerols. A small amount of water is essential for lipase catalyzed rancidity as it is one of the reactants, but triacylglycerol lipase is active at water levels that would completely inhibit most other enzymes, which means that drying a product may not particularly effective in preventing lipase-catalyzed rancidity from taking place. However, if the bran is subjected to a short-term high-temperature heat treatment immediately after milling, the lipase activity is deactivated and stable bran is produced. Work performed (Tao, 1989; Malekian, 1992) on the use of microwave heat energy for rice bran stabilization has shown that microwave energy successfully inactivates lipase, the enzyme partly responsible for the instability of rice bran. Treating bran with microwave heat will not affect the functional properties nor proximate composition. The thermal efficiency of microwave processing of bran is better than conventional heat treatments and the processing time is shorter.

Oxidative rancidity involves a reaction between the lipid and molecular oxygen. The reaction takes place at the double bonds of unsaturated fatty acids and can be accelerated by singlet oxygen, free radicals, metal ions (iron, copper, and

cobalt), light, radiation, and enzymes containing a transition metal prosthetic group such as lipoxygenase (Barnes and Galliard, 1991). Also, the reactions are dependent on fatty acid composition (Nawar, 1985). LOX is found in a variety of plants, particularly legumes, such as soybeans, mungbeans, navy beans, green beans, peas and peanuts; and in cereal such as rye, wheat, oat, barley and corn (Tappel, 1963). Unlike lipase, and like most other enzymes, LOX activity is accelerated by the addition of water to cereal products (Barnes and Galliard, 1991).

LOX specifically oxygenates polyunsaturated fatty acids and/or their esters and acylglycerols containing the cis, cis-1,4 pentadiene double bound system located between carbons 6-10 counting from the methyl terminus (Shastry and Raghavendra Rao, 1975). Under appropriate conditions LOX leads to deterioration of fat-soluble vitamins and essential fatty acids (linoleic and linolenic acid) of oils and fats. It also causes off-flavor and off-odor in food because of its reaction with unsaturated fatty acids. The most reliable method for testing rancidity development is the tedious periodic evaluation of stored samples under actual commercial packaging conditions. A variation of the storage test extends conditions of holding samples at various temperature to accelerate this effect on lipids present. Little research has been done on the kinetics of LOX-catalyzed reactions because of the complicated nature of the system. Although previous studies (Tao, 1989; Malekian, 1992) have shown that microwave processing of rice bran results in inactivating the lipase responsible for hydrolytic rancidity, no information is available in the published literature as to how the deleterious effect of oxidative rancidity on bran can be controlled during storage.

Therefore, the objectives of this study are:

1. To determine the effect of packaging methods (vacuum vs. Ziplock® bags) and storage conditions on lipase activity.

2. To determine factors such as storage time, storage temperature, and packaging methods on lipoxygenase activity.

3. To determine the effect of microwave heat, packaging methods, and storage temperature and conditions on fatty acid and proximate composition in rice bran.

This dissertation involves the stabilization of freshly milled rice bran with microwave heat and packing the samples in Ziplock® bags and vacuum pack and storing the samples in refrigerator and 25°C for 16 weeks and testing the samples for FFA, LOX activity, fatty acid and proximate composition at 4 week intervals.

This dissertation consists of 7 chapters. The first chapter introduces the rice bran as a food commodity and the utilization problems such as rancidity and nutrient losses during stabilization process and storage. The second chapter reviews and updates the status of rice bran composition, health benefits, stability, hydrolytic and oxidative rancidity, and nutrient losses in regard to packaging methods and storage temperatures to extend the shelf-life. Chapter 3 deals with stabilization of rice bran with microwave heat, packing the samples in Ziplock® bags and vacuum pack and storing the samples in the refrigerator (25°C) and room temperature (4-5°C). Determination of free fatty acid (FFA) in terms of % oleic acid as a marker for hydrolytic rancidity is discussed. Oxidative rancidity is investigated thoroughly in chapter 4. Lipoxygenase activity is measured by spectrophotometer at 4 week

intervals and specific activity is being investigated. Nutritional quality is important in considering rice bran as a food commodity. In chapter 5, macronutrients , fatty acid composition of raw and microwave heat stabilized rice bran is being monitored during 16 weeks storage at 4 week intervals. Effect of packaging methods (vacuum verses non-vacuum) and storage temperature (4-5°C verses 25°C) on microwave heat stabilized and raw rice bran is discussed in chapter 6. In chapter 7, a summary, conclusion, and recommendations drawn from investigations reported in sections 2 through 6. The reference citations are included at the end of Dissertation. Appendices include three graphs related to processing steps of rice bran and statistical analysis examples, are reported right after reference citations.

CHAPTER 2
REVIEW OF LITERATURE

Rice Bran Production. Rice is unique among the world's major crops because of its many uses and its capability to adapt to climatic, agricultural, and cultural conditions. Its ability to grow and produce high caloric food values per unit area of all types of land makes rice the world's most important cereal crop (Mikkelsen and de Datta, 1991). Rice bran is a by-product which is produced during the process of milling. The bran constitutes nearly 7- 8.5% of the total grain. The product fractions from standard milling of rice is shown in Figure 1 (Henderson and Perry, 1976). The bran consists of the pericarp, tegmen (the layer covering the endosperm), aleurone, and sub-aleurone (Houston, 1972).

Rice Bran Composition. When bran layers are removed from brown rice during milling, rice bran is produced. Rice bran is rich in nutrients with protein content of 14-16%. The nutritional value of rice bran protein is relatively high, due to the high lysine content, one of the essential amino acids. The reported protein efficiency ratio (PER) is 1.6-1.9, compared to the value for casein of 2.5 (Saunders, 1990). Carbohydrates in rice bran are hemicellulose (8.7-11.4%), cellulose (9-12.8%), starch, and β-glucan (1%).

Rice bran contains 15-23% oil. Three major fatty acids, palmitic (12-18%), oleic (40-50%) and linoleic (30-35%), make up 90% of total fatty acids. Crude rice bran oil contains 3-4% waxes and about 4% unsaponified lipids. Oryzanol and vitamin E, potent antioxidants, are present in rice bran (Saunders, 1985).

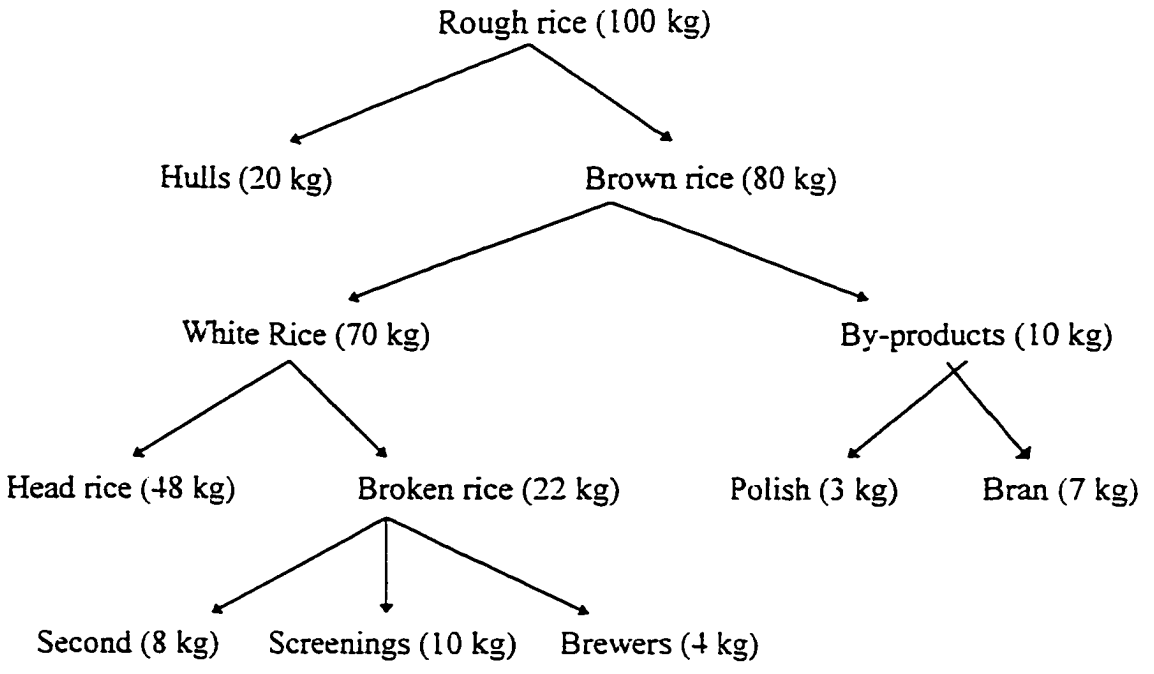


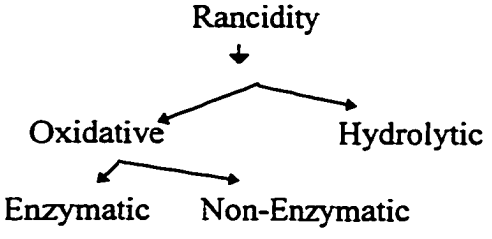
Figure 1. Product fractions from standard milling of rice (Henderson and Perry, 1976)

Rice bran is also rich in B-complex vitamins. The mineral composition of rice bran depends on nutrient availability of the soil in which the crop is grown. Rice bran contains iron (130-530μg/g), aluminum (54-369μg/g), calcium (250-1310μg/g), chlorine (510-970μg/g), sodium (180-290μg/g), potassium (13,200-22,700μg/g), magnesium (8,600-12,300μg/g), manganese (110-880μg/g), phosphorus (14,800-28,700μg/g), silicon (1,700-7,600 μg/g), and zinc (50-160μg/g). Bran contains 80% of rice kernel iron (Lu and Luh, 1991).

Health Benefits of Rice Bran. Nutritional studies in animals and humans have shown a cholesterol-lowering potential for rice bran and rice bran fractions (Seetharamaiah and Chandrasekhara, 1989; Kahlon et al., 1990; Kahlon et al., 1991; Nicolosi et al., 1991; Rukmini and Raghuram, 1991; Newman et al., 1992; Hegsted et al., 1993). Among compounds whose hypocholesterolemic activity has been demonstrated in animal and/or human subjects are rice waxes, oryzanols, hemicelluloses, neutral-detergent fiber fractions, proteins, and oil components (Saunders, 1990). Rice bran can be used as a stool bulking agent (Tomlin and Read, 1988). Diets high in unsaturated fatty acids such as oleic, linoleic and linolenic acid, which are present in rice bran oil, lowered LDL-Cholesterol when replacing saturated fat (Mattson and Grundy, 1985; McDonald et al., 1989).

Utilization of Rice Bran. After the bran layer is removed from the endosperm during milling, the individual cells are disrupted, and the rice bran lipids come into contact with a highly reactive lipase enzyme. Freshly milled rice bran has a short shelf-life because of decomposition of lipids into free fatty acids, FFA, (hydrolytic

rancidity), making it unsuitable for human consumption and the economical extraction of edible rice oil. In rice bran the hydrolysis is catalyzed by endogenous enzyme activity (lipases) and, to some extent, by microbial enzymes if the material is of poor quality (Barnes and Galliard, 1991). The hydrolysis of lipids in rice bran becomes apparent in several ways: off-flavor such as a soapy taste, increased acidity, reduced pH, changes in functional properties, and increased susceptibility of fatty acids to oxidation. The FFA goes under further decomposition (oxidative rancidity) and results not only in free radicals but also bad taste as well as loss of nutritional values. Types of rancidity are be shown according to the flow chart below (Barnes and Galliard, 1991):



Hydrolytic Rancidity. The oil in unmilled paddy rice and brown rice is relatively stable because the lipolytic enzymes are located primarily in the testa and cross cells of the seed coat, while most of the oil is stored in the aleurone cells and germ (Saunders, 1985). During the milling operation this physical separation is disrupted and lipase enzyme comes into contact with neutral fat, causing hydrolysis of fat to FFA and glycerol in the bran.

Types of Lipases. Rice bran contains several types of lipases as well as phospholipases, glycolipases, and esterases (Takano, 1993). Rice bran lipase has a molecular weight of 40 kDa, a pH optimum of 7.5-8.0, and an optimum temperature of 37°C. The enzyme cleaves fatty acid ester bonds at the 1,3-site (Aizono et al., 1971). Phospholipases include phospholipase A1, phospholipase A2, phospholipase B, each acting on fatty acid ester parts and phospholipase C and phospholipase D acting on phosphate part (Takano, 1993). Triacylglycerols (TG), the main component of rice bran lipids, occur as spherosomes. Takano (1993) proposed the decomposition mechanism of rice bran lipids by lipases as follows: phosphotidylcholine, the major component of the spherosome membrane, is decomposed into phosphatidic acid by phospholipase D, and thus, spherosomes are disintegrated, then triglycerols (TG), which are protected by the membrane, come into contact with lipase and its decomposition process begins, causing an increase in free fatty acids.

Lipase Activity. In rice bran oil as FFAs increase, the refining loss for edible oil production increases more rapidly because refining loss is two to three times the percentage of FFA. Refining of the crude oil with more than 10% FFA is considered uneconomical. Rice bran oil normally contains 1.5-2% FFA at milling. Less than 5% FFA is desirable in the crude oil for economic refining purpose (Enochian et al., 1980). The FFAs produced, especially polyunsaturated fatty acids such as linoleic acid (the best substrate for LOX), are subjected to oxidation by LOX. Because FFAs accumulate to unacceptable levels (more than 5%) within a few hours after milling, the lipase enzymes must be inactivated quickly. The value for FFA (% oleic acid) present

is widely used as a quality indicator for fats and oils. The test is based on an alcohol extraction with sodium hydroxide titration for end-point neutralization using m-cresol purple as an indicator (Hoffpauir et al., 1947).

Rice Bran Stabilization. Stabilization or inactivation of lipolytic enzymes in freshly milled rice bran has been of interest to researchers. Many procedures, such as those utilizing pH (Prabhakar and Venktesh, 1986), ethanol vapors (Champagne et al., 1992), and moisture and heat (Saunders, 1985), have been used to inactivate lipase to stabilize rice bran and extend its shelf-life. According to Aizono and co-workers (Aizono et al., 1971; 1976), the rice bran lipases have pH optima of 7.5 to 8.0; if the pH either decreases or increases, the lipase activity decreases. Prabhakar and Venkatesh (1986) showed that the lipase was considerably active in its native state at pH 4.5. The pH of the bran had to be lowered to 4.0 to have low level enzyme activity. Even at pH 4.0 an increase of 3.0-9.3% in free fatty acids occurred after 51 days of storage. Prabhakar and Venkatesh (1986) further concluded that chemical methods are not very efficient in rice bran stabilization. Ethanol vapors caused a slight increase in FFA after 5 days of storage, but 3% of oil was lost (Champagne et al., 1992). The only practical method, which has commercial potential, is heat treatment of freshly milled rice bran (Deikachar, 1974). Depending on the type of heat treatment, the lipases may be either reversibly inhibited or permanently denatured. There are different types of heat stabilization procedures: retained moisture heating (Lin and Carter, 1973), added moisture heating (Saunders, 1985), dry heating in

atmospheric pressure (Loeb et al., 1949), extrusion cooking (Sayre et al., 1982), and microwave heating (Tao, 1989; Malekian 1992).

Microwave Heat Stabilization. Microwave heating is becoming increasingly more popular and important in cooking and food processing. This method of cooking or processing offers conservation of time and energy. The microwave heat treatment for extending the shelf-life of soybean curds was successful (Wu, 1977). Tao (1989) and Malekian (1992) showed that exposure of fresh rice bran samples with 21% moisture content for 3 min inactivates lipase activity (increase in % oleic acid) for 8 weeks. Microwave heat did not considerably affect nutritional quality (proximate analysis), and the functional property of rice bran. In this study, the effect of microwave heat, two different packaging methods (Ziplock® and vacuum packing), and different storage temperatures (5°C and -20°C) for up to 16 weeks on FFA (% oleic acid), LOX activity, and fatty acid composition will be investigated.

Oxidative Rancidity. The reaction of oxygen with unsaturated lipids (LH) involves free radical initiation, propagation, and termination processes (Frankel, 1984). Initiation takes place by loss of a hydrogen radical. The resulting unstable lipid free radicals ($L^{\bullet}$) react with oxygen to form peroxy radicals ($LOO^{\bullet}$). In this propagation process, $LOO^{\bullet}$ react with more LH to form lipid hydroperoxides (LOOH), which are the fundamental primary products of autoxidation (Frankel, 1984).

$$LH \xrightarrow{\text{initiator}} L^{\bullet} + H^{\bullet}$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet}$$

Decomposition of lipid hydroperoxides is a very complicated process, but has biological effects and causes flavor deterioration in fat-containing foods. This decomposition proceeds by homolytic cleavage of LO-OH to form alkoxy radicals LO$^{\bullet}$. These radicals undergo carbon-carbon cleavage to form breakdown products including aldehyde, ketones, alcohols, hydrocarbons, esters, furans and lactones (Frankel, 1982). LOX catalyzes the addition of oxygen to the chain reaction to form hydroperoxides. The complete lipid oxidation is composed of four parts (DeGroot et al., 1975): (a) the activation of enzyme; (b) the aerobic pathway; (c) the anaerobic pathway; and (d) the non-enzymatic pathway. The reactions are similar to those occurring during autoxidation, but LOX can act much more rapidly than autoxidation and is more specific in terms of end-products.

Isolation and Purification of Lipoxygenase. LOX extraction and purification (except soybean LOX-1) is difficult. These enzymes are present in different isomeric forms (O'Connor and O'Brien, 1991). As a result, conducting studies with these enzymes are very difficult. Theorell et al. (1947) were the first to prepare crystalline LOX from soybean. Almost every protein purification method can be used, ranging from conventional techniques such as ammonium sulfate precipitation, gel filtration, ion exchange, and hydroxyapatite chromatography, to more recently developed chromatographic techniques, such as affinity chromatography and high performance liquid chromatography (O'Connor and O'Brien, 1991).

Lipoxygenase Activity Determination. Some of the complications in the assay of LOX activity include the low solubility of the fatty acids in an aqueous

system, purity of fatty acids, inhibition by fatty acid substrate, aerobic and/or anaerobic reactions, effect of pH and temperature on positional specificities, and inactivation of enzyme during reaction (Whitaker, 1991). Several methods can be used to measure LOX activity. The most commonly used are the manometric techniques based on the measurement of oxygen uptake by the substrate, and spectrophotometric assay in which the increase in extinction brought about by the formation of the conjugated diene from linoleate is followed (Ben-Aziz et al., 1970). Some of the methods used are as follow:

(1) The rate of hydroperoxide formation can be measured by conversion of $Fe(CNS)_2$ to $Fe(CNS)_3$. In this method aliquots must be removed from the reaction for color development. The colored $Fe(CNS)_3$ is unstable and absorbency must be read soon after mixing (Whitaker, 1991).

(2) Enzyme activity can be assayed continuously by measuring oxygen uptake (Polagraphic method). Mitsuda et al. (1967b) used an oxygen electrode as a detector for measuring oxygen concentration in the reaction medium. In this method the atmospheric oxygen must be continuously controlled which makes the measurement of oxygen consumed by enzymes very difficult.

(3) LOX activity can be assayed by simultaneously measuring hydroperoxides and pentane production by polagraphy. The amount of pentane produced is proportional to the quantity of hydroperoxide formed (O'Connor and O'Brien, 1991).

(4) High Performance Liquid Chromatography (HPLC) is used for separation of hydroperoxides (Yamamoto et al., 1980; Andre and Funk, 1986). In this method the

stereochemistry of LOX-derived products of polyunsaturated fatty acid metabolism is determined. This method involves derivitization of the compound of interest, so it is time-consuming and not cost-effective.

(5) Gas Chromatography-Mass Spectra (GC-MS) can be used to determine the correlation between oxidative deterioration of unsaturated lipids and n-hexanal during storage of brown rice, and to separate products of linoleic acid and arachidonic acid (Shin et al., 1986; Lehmann et al., 1992; Lehmann et al., 1995).

(6) Rate of product formation can be measured continuously in a recording spectrophotometer at 234 nm. The increase in absorbency is due to the formation of conjugated double bonds in the fatty acid hydroperoxide (Whitaker, 1991). This method is one of the best and most commonly used assays for this enzyme. The assay is continuous, reproducible, and specific (Surrey, 1964; Ben-Aziz et al., 1970; St.Angelo et al., 1972; Hafez et al., 1985b; Gibian and Vandenberg, 1987). In this procedure linoleic acid (C18:2) is used as an assay substrate.

Lipoxygenase Classification. LOX in soybean is classified into three types according to the positional specificities of the hydroperoxide (HPOD) produced (Galliard and Chan, 1980). When linoleic acid is used as the substrate, the first type of LOX (soybean LOX-1) specifically produces 13-HPOD. Rice and rice bran do not have this type of LOX. The second type of LOX produces 9-HPOD and 13-HPOD in equal amounts. Rice LOX-1 and LOX-2 belong to this type (Ida et al., 1983). The last type of LOX specifically produces 9-HPOD. Rice LOX-3 has this positional specificity (Yamamoto et al., 1980; Ida et al., 1983) like maize and potato LOXs

(Hiroyuki et al., 1986). According to Shastry and Raghavendra Rao (1975), unfractionated rice bran extract contained three distinct bands on disc-gel electrophoresis with LOX activity and was stable for 15 days at 3-5°C.

LOX-3 is the major enzyme in rice grain with a molecular weight between 93 and 89 kDa (Hiroyuki et al., 1986). The purified enzyme (LOX-1) has a specific activity of 280 μmol O_2 formed/min per mg of protein. The enzyme is a single polypeptide chain and some similarities in the amino-acid composition exist between rice, soybean and pea LOX. This enzyme is a non-heme iron protein and the purified enzyme contains 73 Fe atoms per molecule. LOX in its native (resting) form is colorless. LOX-3 can be inactivated by SH compounds, such as cysteine and glutathione (Hiroyuki et al., 1986). In spite of many reports of LOX components in higher plants, the enzymatic properties of these components, except those of soybean, have not been extensively studied. This is because most LOXs are not stable during purification, in contrast to the soybean LOXs (Galliard and Chan, 1980). In a recent study by Skrzypezak-Jankun et al. (1997) the structure of soybean lipoxygenase L3 was studied and compared with its L1 isoenzyme. They showed a possible explanation for a stringent stereo-specificity of catalytic products in L1 which produces mostly 13-hydroperoxide versus the lack of such a specificity in L3 which produces a mixture of 9- and 13-hydroperoxide and their diestereoisomers.

Mechanism of Lipoxygenase Reaction. LOX (linoleate: oxygen oxidoreductase E.C. 1.13.1.13) catalyzes the oxidation of methylene-interrupted unsaturated fatty acids and their esters such as linoleic and linolenic acids. LOX is

very important to food scientists for a number of reasons. LOX can effect color, flavor (off-flavors in frozen vegetables, stored cereals, high-protein foods), and nutritive properties. For example, there is destruction of vitamin A, loss of essential polyunsaturated fatty acids, and interaction of enzymatic product with some essential amino acids that lower the quality of protein (Richardson and Hyslop, 1985).

Lipase and lipoxygenase enzymes, both of plant and animal origin, generally are activated when tissue is disrupted or injured. Sequential enzyme action on lipids starts with the release of fatty acids (lipolytic enzymes). Among the FFA, the polyunsaturated are oxidized to fatty acid hydroperoxides by lipoxygenase (see equation below).

$$\text{Unsaturated fatty acid} + O_2 \xrightarrow{\text{lipoxygenase}} \text{peroxide derivative of unsaturated fatty acids}$$

The most typical substrate are naturally occurring isomers of three essential fatty acids: linoleic, linolenic, and arachidonic acids. The next step leads to decomposition or enzyme conversion of hydroperoxides into a number of oxygenated fatty acids (Gardner, 1979). Hexanal is generally accepted as one of the major components responsible for off-flavor developing in long-term-stored rice kernels (Yamamoto et al., 1980).

LOX is an enzyme that imitates the autoxidation of polyunsaturated fatty acids, except that LOX is selective for the type of substrate it oxidizes and how the substrate is oxidized. In soybeans, several iso-enzymes have been found. LOX-1 is the most thoroughly investigated species and linoleic acid is the best substrate for this

enzyme. Hamberg and Samuelsson (1967) concluded that a cis, cis-1,4-pentadeine moiety having a methylene group (a methylene between two double bonds) located at the eight carbons from the terminal methyl end is necessary for oxidation of the substrate by soybean LOX. Such fatty acids are oxidized only at the 6th carbon, except linoleic acid (LH) is oxidized at both 6th and 10th carbons. With linoleic acid, the principal oxygenation products are optically active 9 and 13 hydroperoxide isomers (Theorell et al., 1947). Iron present in the LOX appears to be involved in electron transfer during the incorporation of O_2 into unsaturated fatty acids containing cis,cis-1,4-pentadiene system (see as follows).

cis cis
CH3-(CH2)4-CH=CH-CH2-CH=CH-(CH2)7-COOH
Linoleic Acid

$$\text{Linoleate} + O_2 \xrightarrow{\text{LOX}} \text{13-hydroperoxyoctadeca-9, 11,dienoate}$$

LOX must be in the oxidized (Fe^{3+}) form for the oxidation reaction to proceed. Then the oxidized form of LOX can catalyze the stereospecific removal of hydrogen from the C-11 methylene group of linoleic acid (C18:2) or linolenic acid (C18:3) (O'Connor and O'Brien , 1991). The 11-pro-S-hydrogen is removed, a C-13 radical is formed, and LOX is reduced to the Fe^{2+} form. Under anaerobic or aerobic conditions the reaction continues and the hydroperoxides may form other products (Figure 2. Gardner, 1988). Hydroperoxide lyase produces aldehydes and aldehyde acids from hydroperoxide; flour-isomerase produces epoxyhydroxyene fatty acids that are hydrolyzed to trihydroxyene fatty acids; hydroperoxide isomerase produces α-ketol

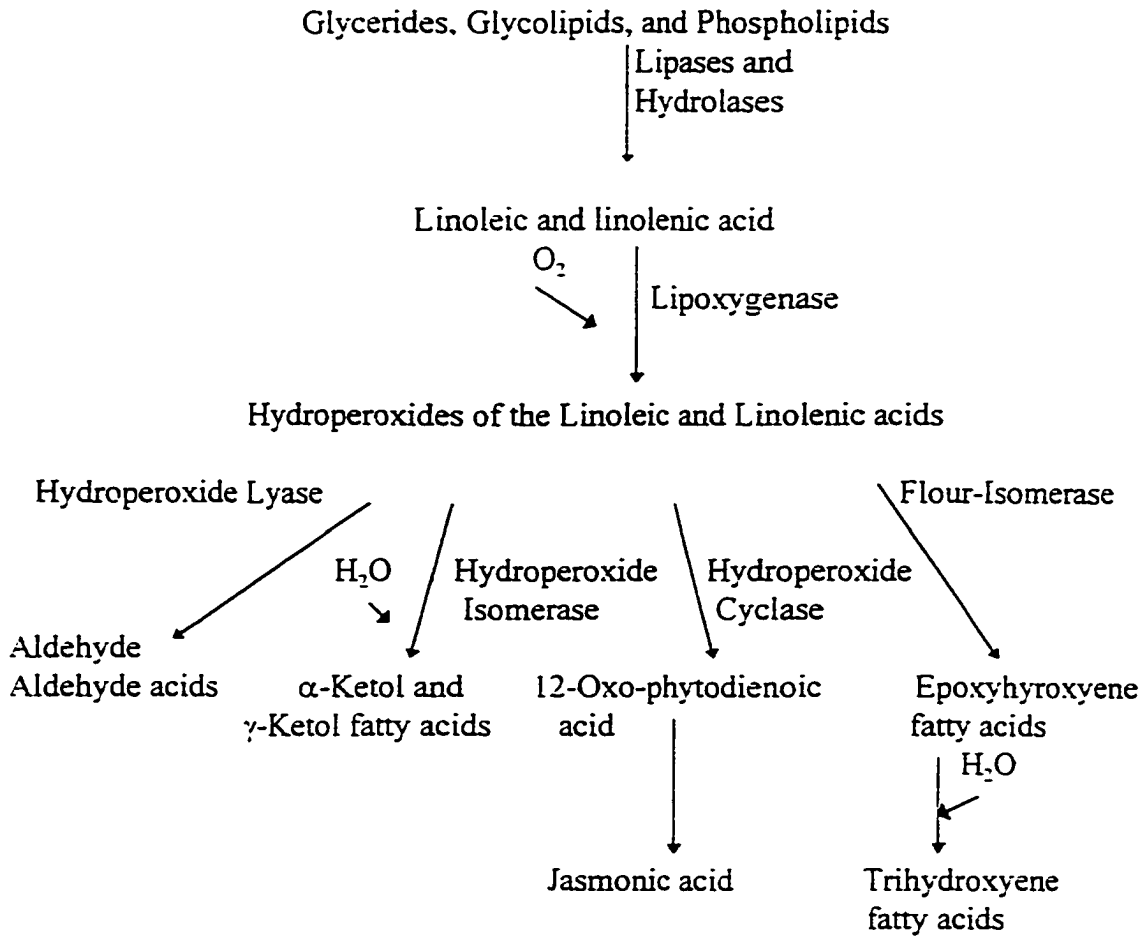


Figure 2. Products formed enzymatically from linoleic and linolenic acids in plants (Gardner, 1988).

and γ-ketol fatty acids; and hydroperoxide cyclase produces 12-oxo-phytodienoic acid, a precursor of jasmonic acid. Jasmonic acid is a plant growth hormone that regulates plant responses to wounding and pathogens and, in addition, is an inducer of tuberization in the potato (Royo et al., 1996). The non-enzymatic oxidation of linoleic acid produces as many as 74 compounds (3 hydrocarbons, 25 aldehydes, 2 ketones, 6 alcohols, 12 acids, 19 esters, and 7 miscellaneous compounds (Grosch, 1987).

Inhibition and Inactivation of Lipoxygenase. LOX-produced off-flavors are a significant potential problem in products containing lipids. Many researchers have been working on optimizing the condition necessary for the inactivation of LOX in such products. Methods being investigated include addition of antioxidant, pH adjustment, and heat (O'Connor and O'Brien, 1991).

LOX activity in a model system is inhibited by various antioxidants e.g., (pyrocatechol, homocatechol, propyl gallate, nordihydroguariaretic acid, resorcinol, phioroglucinol, hydroquinone, butylated hydroxyanisole, and various flavonoids and related compounds) because of the nature of the enzyme (Takahama, 1985; O'Connor and O'Brien, 1991). Hydrogen peroxide (H_2O_2) inactivates soybean LOX1 irreversibly (Mitsuda et al., 1967). Heat treatment can affect protein solubility and adversely change the functional properties of soy products. Brown et al. (1982) inactivated LOX by 99% at a temperature of 91°C and above while 70% of protein solubility was retained. They adjusted soybean moisture to 16.3% with pH 9.8 buffer and then heated the samples with steam for 10 seconds. Graveland (1970) showed that oxidation of linoleic acid in flour-water suspensions leads to production of two

isomeric hydroxy-octadecadienoic acids. He noticed that defatting flour with petroleum ether leads to an increase in production of optically active 9 and 13 hydroperoxide isomers. Defatting did not affect the LOX activity. Williams et al. (1986) reported that LOX was the primary cause of development of off-flavors in English green peas and green beans. LOX was responsible for aroma changes defined as unripe, banana, grassy, straw, ammonia, and partly responsible for the sour component. LOX was also responsible for the overall aroma changes. Pea and green bean LOX were more heat sensitive than peroxidase at 60° C for 10 min. Therefore, a less severe heat treatment was required to inactivate LOX in English green peas and green beans. According to Ganthavorn et al. (1991), LOX in asparagus tips was more heat stable than peroxidase. Therefore, a heat treatment (50°, 60°, or 70° C for 10 min.) sufficient to inactivate peroxidase may not be sufficient to inactivate LOX. Differences in heat stability of LOX from asparagus and green peas indicate the importance of independently evaluating enzyme stability in different vegetables.

Yamamoto et al. (1980) observed a relatively high lipoxygenase activity in the unfractionated fresh rice bran which was removed from brown rice. Dhaliwal et al. (1991) studied the LOX changes in milled rice obtained from different varieties of patties stored for 1, 6, and 12 months with two different moisture levels. They concluded that LOX activity was not altered with drying. Sekhar and Reddy (1982) concluded that since LOX acts on polyunsaturated fatty acids, such as linoleic acids which are present in up to 40-45% of the total fatty acids in rice, it can be assumed that the varieties with lower activities of this enzyme may have better storage qualities.

Esaka et al (1986) found that microwave heat may be effective for inactivation of LOX and trypsin inhibitor in whole soybean. The microwave heating could be considerably effective in inactivating the LOX and trypsin inhibitors of whole winged bean seeds and in increasing the rate of water absorption of the seeds (Esaka et al., 1987). Wang and Toledo. (1987) concluded that microwave heat treatment of soybeans at their natural moisture content (8.7%) for 4 min could provide suitable material for soy milk processing. Esaka et al. (1987) reported that LOX was completely inactivated in winged beans by microwave heating for 3 min. Soaking of the seeds before microwave heating decreased the heating time needed to inactivate the enzyme.

The microwave processing of rice bran results in inactivating lipase responsible for hydrolytic rancidity for a storage period of 8 weeks (Tao, 1989; Malekian, 1992). No information is available in the published literature as to how the deleterious effect of oxidative rancidity on bran can be controlled during storage. Furthermore, there is no report on the effect of microwave heat on LOX activity in rice bran. LOX, which is not generally associated with oil degradation, is the enzyme which promotes oxidation of unsaturated fatty acids in rice bran (which is a good source of edible grade oil) responsible for rancid flavor and odors. Bran, after proper stabilization, is a good source of calories, essential fatty acids, and nutritionally interesting products such as tocopherol and ferulic acid derivatives. Protection of the unsaturated fatty acids of bran during storage, and consequently of the nutritional

value of rice bran, promises wider markets to rice millers and farmers, in addition to providing a healthy food product to consumers.

This study was undertaken to determine suitable, convenient, simple and accurate methods to stabilize rice bran, to extend the shelf-life of rice bran by using proper packaging methods and materials, and to prevent macronutrient losses during processing and storage which are present in rice bran. Specifically, this study determined the effect of microwave heat on LOX activity, lipase activity, and fatty acid composition, considering the packaging methods (Ziplock® bags and vacuum packing) and storage conditions (room temperature and refrigerator) for up to16 weeks. Figure 3 shows the schematic diagram of this project, which consists of:

1. The hydrolysis rancidity by lipase activity in terms of increase percent of free fatty acids in microwave stabilized rice bran compared to control determined by titration;

2. The oxidative rancidity by lipoxygenase activity in terms of increase in absorbency A_{234nm} /min determined by spectrophotometer;

3. The fatty acid and proximate composition of the rice bran;

4. The effect of packing methods and storage temperature on the shelf-life of stabilized bran. Two packing methods, one using a regular Ziplock® plastic bag and the other using vacuum packing, were used, since these are the most commonly used commercial methods for packaging foods.

Nutritional data such as fat, protein, moisture, ash, and total carbohydrate content were obtained by proximate analysis of all bran samples at 0 weeks and at the end of 16-week storage periods in vacuum and Ziplock® packed samples.

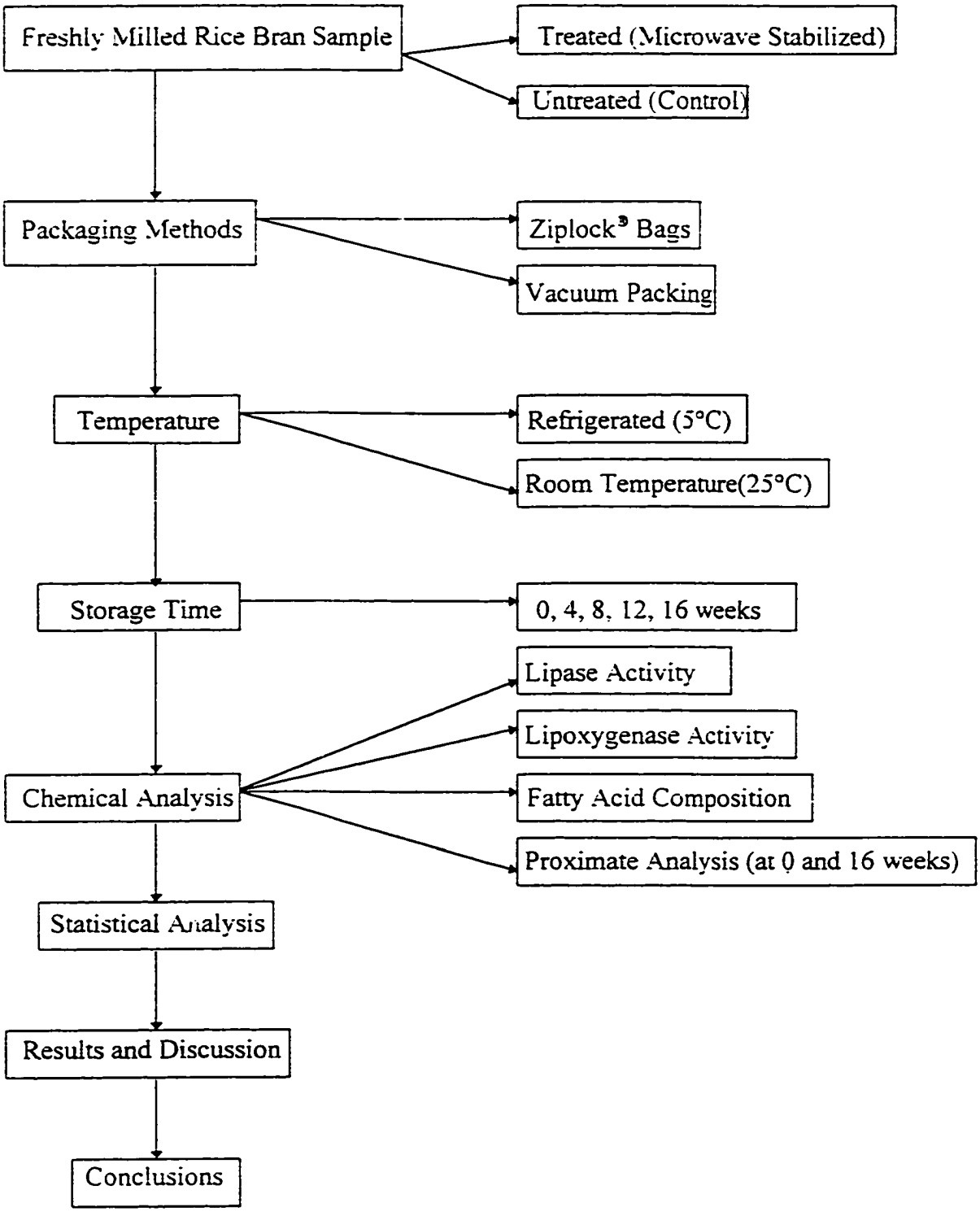


Figure 3. Experimental plan to evaluate rice bran treatment and storage condition on hydrolytic and oxidative rancidity and nutrient losses.

CHAPTER 3
PREVENTION OF HYDROLYTIC RANCIDITY IN RICE BRAN DURING STORAGE

Introduction. Rice bran is a by-product which is produced during the process of milling. The bran constitutes nearly 7-8% of the total rice grain (Henderson and Perry, 1976). The bran consists of pericarp, tegmen (the layer covering the endosperm), aleurone, and sub-aleurone (Houston, 1972). Rice bran is rich in nutrients. When bran layers are removed from the endosperm during milling process, the individual cells are disrupted, and the rice bran lipids come into contact with highly reactive lipase enzymes. Lipases, both endogenous to the bran and of microbial origin, initiate hydrolytic deterioration of kernel oil (Champagne et al., 1992). Most enzymes are effective in aqueous systems where both the enzyme and substrate are soluble. In the case of lipase, the substrate is insoluble in water and lipase is active at the oil-water interface (Laning, 1991). Freshly milled rice bran has a short shelf-life because of decomposition of lipids (triaclyglycerols) into free fatty acids by lipases, making it unsuitable for human consumption or economical extraction of edible oil (Barnes and Galliard, 1991). However, if the bran is subjected to a short-term high temperature heat treatment immediately after milling, the lipase activity is inactivated and stable bran is produced. Work performed (Tao, 1989; Malekian, 1992) on the use of microwave heat energy for rice bran stabilization has shown that microwave energy successfully inactivates lipase, and thereby, extends the shelf-life. raw samples vacuum packed had higher increase in FFA than samples packed in Ziplock® bags after 8 weeks of storage in the refrigerator (4-5°C). FFA in microwave heat stabilized

samples did not increase significantly in both packaging methods (Malekian, 1992) during 8 weeks of storage in the refrigerator (4-5°C). Malekian (1992) concluded that microwave heat could inactivate lipase activity and vacuum packing did not show any advantage over Ziplock® bags. Sharp and Timme (1986) concluded that FFA level for samples packed in a bag was lower than levels for vacuum packed brown rice samples over 9 months of storage time stored at 22°C and 38°C.

The present study was undertaken to determine the effect of microwave heat on lipase activity in two different packaging methods (vacuum verses non-vacuum) and two different storage temperatures storage (4-5°C verses 25°C) during 16 weeks of storage. The specific objectives were:

1. To study the effect of microwave heat on rice bran lipase activity.
2. To study the effect of packaging methods (vacuum packing verses Ziplock® bags) on rice bran lipase activity during 16 weeks of storage.
3. To study the effect of temperature (25°C versus 4-5°C) on rice bran lipase activity during 16 weeks of storage.

Materials and Methods .

Rice Bran Collection. Rice variety 'Lemont' (long grain), cultivated at the Louisiana Rice Experiment Station, Crowley, Louisiana was used for this experiment. The rice samples were dehusked and milled (friction type) by a Satake milling system (Satake USA, Houston, TX) at the Biological and Agricultural Engineering Department at Louisiana State University. Rice bran was collected in a barrel lined with a black plastic bag. Dry ice was added continuously to the rice bran in the barrel

during milling to prevent the hydrolysis of fatty acids by lipase activity. The bag was tied tightly and was delivered (within 15 min) to the Pennington Biomedical Research Center (PBRC) laboratory. The bags were placed in the ultra freezer (-78 to -80 C°) until the day of sample preparation (within 10 days). On the day of sample preparation, the samples were sieved with a 20 mesh siever in order to remove broken pieces of rice and husks. A thermometer was placed in rice bran samples to monitor the temperature (0-2°C) during the sieving process.

Microwave Heat Stabilization. One-hundred-fifty g per batch of raw rice bran were stabilized with a 850 Watts power at 2450 MHz Sharp Carousel microwave oven (Model R3A96, Sharp Electronic Corporation, NJ). The microwave chamber was heated before the stabilizing process by running the microwave at high for 3 min. The moisture content of raw rice bran samples was adjusted from initial 7.0 % to 21 % based on wet basis by using the calculation below:

$$150g \times 0.07 = 10.5$$

$$10.5 + X = 0.21(150g + X)$$

$$10.5g + X = 31.5g + 0.21\,X$$

$$X = 26.58 \text{ ml}$$

Therefore, 26.58 ml of deionized (DI) water was added to 150 g of rice bran sample to obtain 21% moisture content; the percentage of moisture content has been optimized in previous studies (Tao, 1989; Malekian, 1992). The sample was mixed thoroughly with a stainless steel spatula to make sure that the water was evenly distributed. Each sample was placed in a Ziplock® one gallon storage bag (Hefty One Zip multi purpose

storage bag Gallon size, Mobile Chemical Co., NY), and the bag was sealed until the end of microwave heating and spread out evenly to a thickness of 0.5 cm. The sample was heated at 100% power for 3 min. At the end of 3 min, the microwave door was opened and a hole was made in the bag so that a thermometer could be inserted into the sample. The temperature of the sample was 107 ± 2° C. The sample was cooled to room temperature (25°C). This was repeated until there was sufficient bran stabilized with microwave heat for the experiments. The samples were stored in an ultra freezer (-78 to -80°C) until the day of packaging .

Packaging and Storage of Rice Bran. Microwave heat stabilized and raw rice bran were divided in half, and from the first half, ten representative samples, each weighing 70-75g, were packed in polyethylene Ziplock® bags (Hefty One Zip multi purpose storage bag, Quart size, Mobile Chemical Co., NY), yielding a total of 20 bags. The bags were marked for storage time of 0, 4, 8, 12, and 16 weeks.

The other half were placed in an ice box, covered with dry ice, and delivered to the LSU Animal Science Department to be vacuum packed. The temperature of the room was 4-5° C. Ten representative samples, each weighing 70-75g, prepared from raw and microwave stabilized bran, were placed in non-permeable vacuum bags and vacuum packed. The vacuum machine used was TurboVAC (Model # SB600 Howden Food Equipment, Netherlands). The bags were marked for storage time of 0, 4, 8, 12, or 16 weeks. The samples were brought back to the PBRC laboratory in the ice box. All bags were also marked for FFA, fatty acid composition, LOX activity, and proximate analysis (at 0 and 16 weeks of storage). Half of the bags (20 bags total, 5 of

each treatment) were stored at 4-5°C and the other half (20 bags) at 25°C. Room temperature and the temperature in the refrigerator were monitored and recorded daily. Figure 15 shows the schematic of rice bran processing and storage. Approximately 2,450g rice bran was used for this experiment.

Free Fatty Acid Determination. Free fatty acids were determined in duplicate using the method of Hoffpauir et al. (1947) modified to use m-cresol purple indicator instead of phenolphthalein as an indicator. Alcoholic sodium hydroxide was prepared by adding one pellet (0.0894-0.1035 g) of NaOH (#3722-01, J.T. Baker Inc., NJ) to 500 ml of absolute ethanol in a flask. The top was covered tightly and the mixture was mixed on a stirrer/hot plate for 30-60 min or until the sodium hydroxide pellet was completely dissolved. A stock solution of m-cresol purple was prepared by dissolving 0.1 g of m-cresol purple (#C-1393, Sigma Chemical Co., MO) in 24 ml of 0.01 N NaOH solution, and was diluted to 250 ml with DI water. The working solution of m-cresol was prepared by mixing 1 ml of stock solution with 100 ml of absolute alcohol. Approximately 1 ml of alcoholic sodium hydroxide was added by titration to the m-cresol purple working solution to reach a grayish purple endpoint. To standardize alcoholic NaOH, 1 ml of 0.1 N hydrochloric acid was added to 25 ml of m-cresol purple working solution and 10 ml of petroleum ether in a 250 ml erlenmeyer flask. This solution was titrated with the alcoholic sodium hydroxide to a purplish gray endpoint. The concentration of alcoholic sodium hydroxide was calculated by dividing 0.1 by the volume of the alcoholic sodium hydroxide used for the titration.

The blank was determined by titrating 25 ml of m-cresol purple working solution plus 10 ml of petroleum ether with the alcoholic sodium hydroxide.

The fat was extracted from samples with a Soxtec System HT apparatus (Model 1043 Extraction Unit, Perstorp Analytical Inc, MD). The extracted fat was dissolved in 10 ml of petroleum ether (fat solution) in a flask, then 25 ml m-cresol purple working solution was added to this flask. The content of the flask was titrated with alcoholic NaOH. Percent FFA was determined using the calculation below:

% FFA = (ml titrated - ml blank) x 28.2 x concen. alc. NaOH / lipid weight (g)

ml titrated = the ml of alcoholic NaOH which was used to change the color of the above solution (sample solution) to grayish purple at the endpoint.

ml blank = the ml of alcoholic NaOH which was used to change the color of blank solution to grayish purple at the endpoint.

28.2 = multiplication factor obtained by multiplying the molecular weight of oleic acid (282.0) times the percentage and divided by 1000 (ml in 1 liter of solution).

concen.alc. NaOH = 0.1 / ml of NaOH used to standardize.

Statistical Analysis. A completely randomized deign was used. To study the main effect for each factor, three factor factorial (2x2x2) and four factor factorial (2x2x2x4) arrangements (Table 9 in Appendices) were used for each variable. To compare the mean of the results (Table 10 in Appendices), the Student-Newman-Keuls (SNK) test was done (generally this test declare more significant differences than other tests such as Turkey's procedure. A statistical analysis of variance (ANOVA) was performed on all values using the Statistical Analysis System (SAS®) program version

6.12 (SAS, 1997). Differences were considered significant when means of compared sets differed at the $p < 0.05$ level of significance.

Results and Discussions. The effects of microwave heat on bran stability in terms of increase in free fatty acids (% of oleic acid) in raw (R) rice bran packed in vacuum packs (V) and Ziplock® bags (Z), stored at room temperature (RT) and refrigerator (Ref) during 16 weeks of storage are shown in Figures 4 and 5. The FFA level in raw rice bran increased from an initial value of 2.5% to 34.4% and 38.8% in the 4th week of storage, respectively, in RZRT and RVRT with a significant difference ($p < 0.05$) (Figure 4). The FFA level reached 8.9% and 9.3% in RZRef and RVRef, and there was no significant difference between these two levels (Figure 5). At 8 weeks of storage, FFA reached 38.6% and 46.9% for RZRT and RVRT, respectively (Figure 4). These differences were significant ($p < 0.05$) while there was no significant change in FFA in RZRef and RVRef (13.4% and 17.4%) (Figure 5). After 12 weeks the FFA level increased significantly ($p < 0.05$) to 42.0%, 44.9%, 15.4%, and 19.0% for RZRT, RVRT, RZRef, and RVRef, respectively (Figures 4 and 5). At the end of storage period (16 weeks), the FFA level reached 48.0% and 54.3% for RZRT and RVRT, and 19. 5% and 25.4% for RZRef and RVRef. These increases were significant ($p < 0.05$) from the 0 week storage time. The rate of FFA formation was even faster in vacuum-packed samples after 8 weeks of storage.

Figures 4 and 5 show the comparison of the increase in FFA% in microwave heat stabilized (M) bran in two different packaging (Z and V) and stored in two different temperatures (RT and Ref) during 16 weeks of storage.

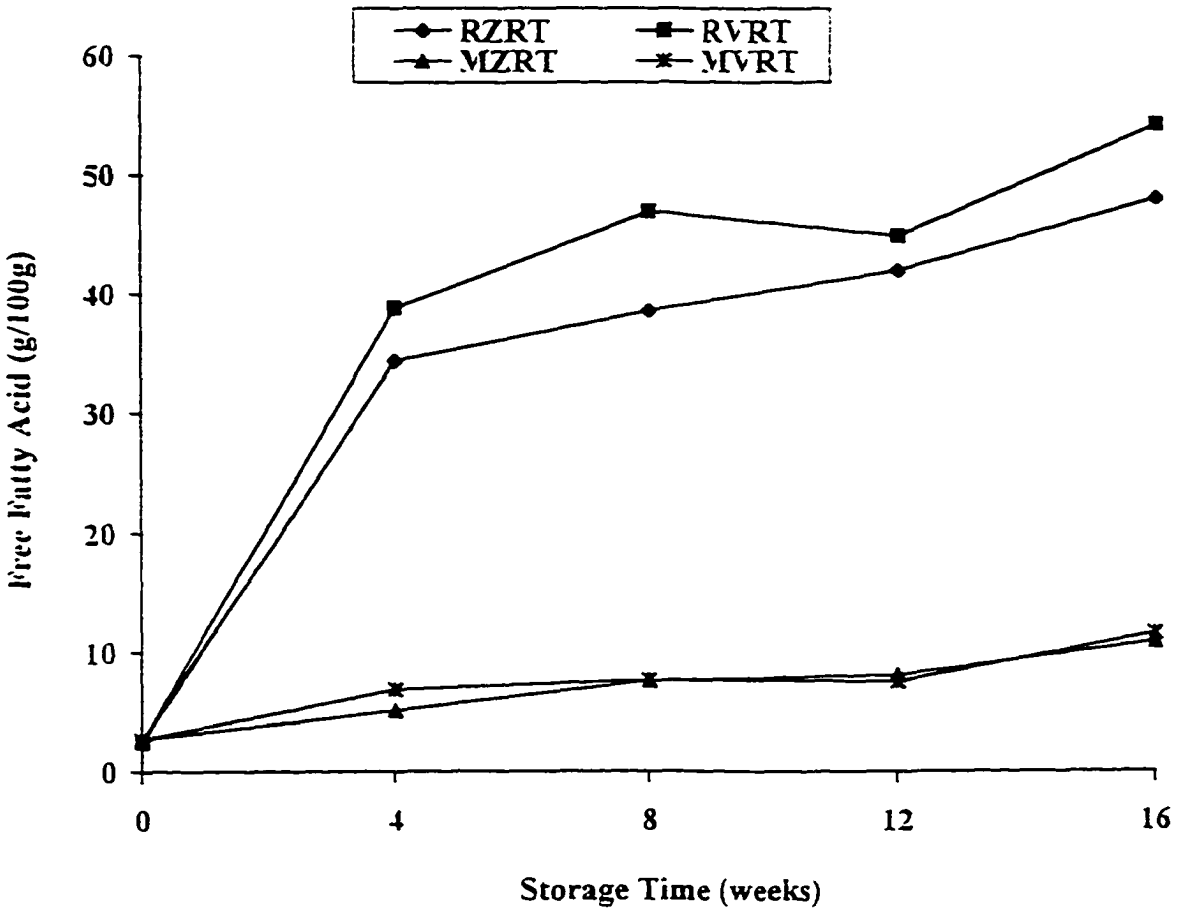


Figure 4. Free fatty acid changes in raw (control) and microwave heat stabilized rice bran packed in Ziplock® bags and stored at room temperature (25°C). Individual values, means and standard deviations presented in Table 13 in the Appendices. R= raw, M= microwave heat stabilized, Z= Ziplock® bags, V= vacuum packed, RT= room temperature

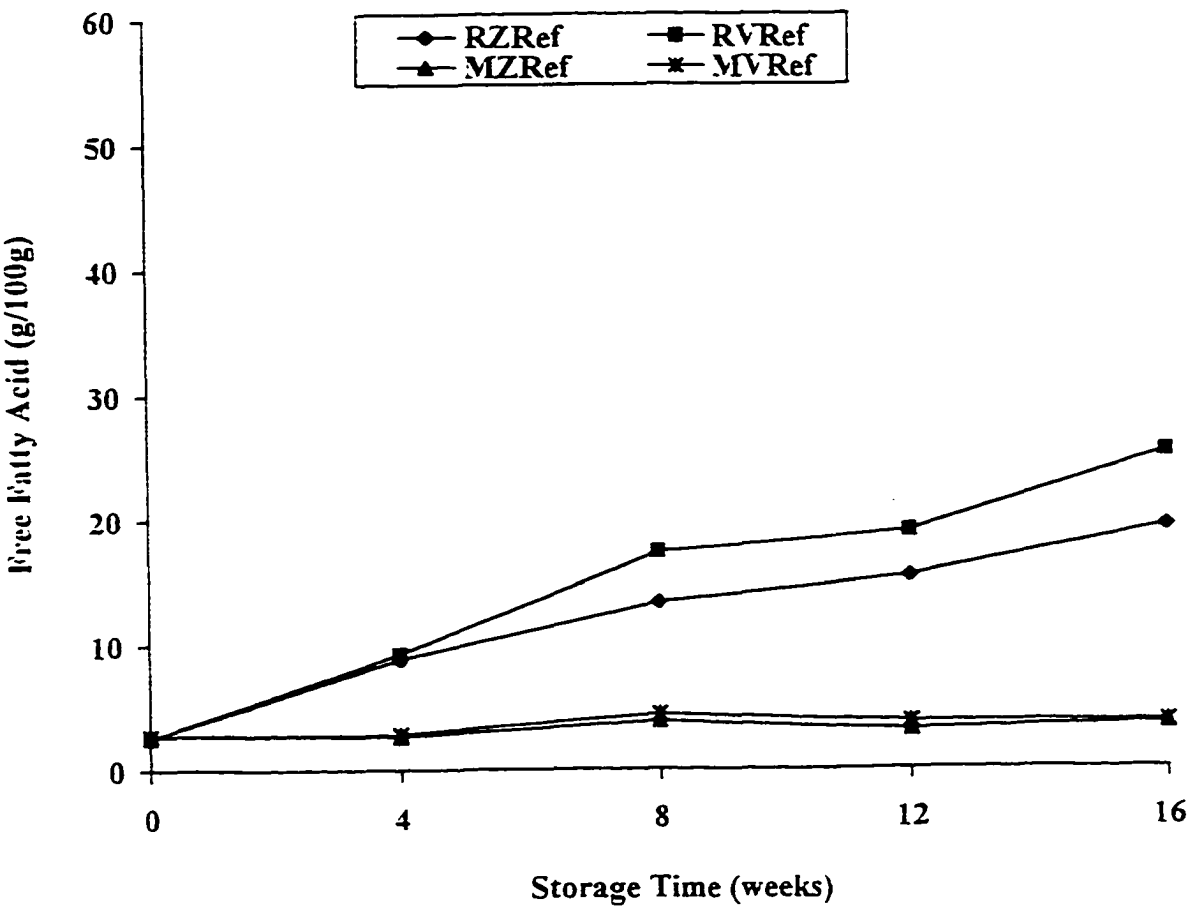


Figure 5. Free fatty acid changes in raw (control) and microwave heat stabilized rice bran packed in Ziplock® bags stored in the refrigerator (4-5°C). Individual values, means and standard deviations presented in Table 14 in the Appendices R= raw, M= microwave heat stabilized. Z= Ziplock® bags, V= vacuum pack, Ref= refrigerator

The FFA level increased from initial value of 2.8% to 5.2%, 6.9%, 2.7%, and 2.9% at the end of 4 weeks, and to 10.9%, 11.6%, 3.6%, and 3.7% at 16 weeks of storage period for MZRT, MVRT, MZRef, and MVRef, respectively. The FFA changes in MZRef and MVRef were not significant ($p < 0.05$) and vacuum packing did not provide superior effect in reducing FFA in rice bran over the non-vacuum Ziplock®.

Figure 4 also shows the increase in FFA for all samples (packed in Ziplock® bags and vacuum packed) stored at room temperature. After 16 weeks, the increase in FFA is highly significant ($p < 0.001$) from the initial FFA content of 2.5% to 48.0% for RZ and 54.3% for RV for untreated raw rice bran and from 2.8% to 10.9% for MZ and 11.6% for MV for microwave stabilized rice bran. Figure 5 shows the FFA increase in samples packed in Ziplock® bags and vacuum packages stored in the refrigerator. The increase in FFA level in raw rice bran samples stored in the refrigerator was from 2.5% to 25.4% for RV and 19.5% for RZ with a significant difference ($p < 0.05$) between samples stored in Ziplock® bags and samples vacuum packed. Microwave heat stabilized rice bran samples showed an increase from initial FFA level of 2.8% to 3.6%for MZ and 3.7% for MV which was not significant, and there was no significant difference in FFA content in samples packed in Ziplock® bags and samples vacuum packed.

The increase in FFA content of raw rice bran is similar to the results obtained by other researchers (Saunders, 1985; Tao, 1989; Martin et al., 1991; Champagne et al., 1992). FFA rose rapidly in raw rice bran, exceeding 50% by 16 weeks (112 days) of storage in vacuum packed samples stored at room temperature. This is typical of

rapid development of hydrolytic rancidity in unstabilized rice bran, which makes this product unsuitable for human consumption (Martin et al., 1993; Tao et al., 1993). Previous studies (Tao, 1989: Malekian, 1992) has shown that FFA content of microwave heat stabilized rice bran increased from 4.0% to 4.9% in long grain rice bran (Lemont) and from 4.6% to 6.2% in medium grain rice bran (Nato) during 4 weeks of storage. In untreated bran the FFA content changes ranged from 4.0% to 68.3% and 4.6% to 56.8% for long and medium grain, respectively. Malekian (1992) showed that the FFA level, in raw rice bran samples placed in Ziplock® bags and vacuum pack and stored in the refrigerator, increased from an initial value of 3.7% to 22.2% and 26.7%, respectively, and there was a significant increase between samples vacuum packed and samples packed in Ziplock® bags (Malekian, 1992). These findings confirms the results obtained in this study. FFA content in microwave heat stabilized bran samples stored in the refrigerator (4-5°C) increased from an initial value of 3.2% to 3.9% regardless of packaging methods.

Interaction between storage temperature and packaging (Figures 4 and 5) showed that the percent FFA in samples from both packaging methods increased regardless of the storage temperatures (4-5°C and 25°C). The FFA levels for both packaging methods were lower when the samples were stored at 4-5°C. Although storage at 25°C greatly increased the FFA level of the Ziplock® bag samples with increase of storage time, the increase was greater in the vacuum packed samples (Figure 4). Sharp and Timme (1986) noticed the same pattern in brown rice stored in bags and vacuum packed samples. This could be because the removal of air and

oxygen by vacuum processing which in turn activates anaerobic microorganisms with higher lipolytic enzyme activity present in raw rice bran. Lipases both endogenous to the bran and of microbial origin, initiate hydrolytic deterioration of kernel oil (Champagne et al., 1992). Surface damage during dehulling disrupts the aleurone and germ (where oil is located) and lipase-producing mold and bacteria found on kernel surfaces would interact with bran oil (Champagne et al., 1992) and the FFA increases.

In this study, in microwave heat stabilized rice bran, the FFA level increased slightly above 10% (10.9% and 11.6%) in samples stored at room temperature (25°C) (Figure 4) and below 5% for samples stored in the refrigerator (4-5°C) (Figure 5) after 16 weeks of storage. There was not a significant difference in FFA content between vacuum packing vs. Ziplock® bags. This could be because of microwave heat destroyed bacteria (specially anaerobic) present in rice bran. Bran oil with an excess of 10% percent FFA and bran with more than 5% FFA is considered unsuitable for human consumption (Tao et al., 1993). The rate of FFA formation in bran or brown rice flour is high. About 30% of the oil can be converted to FFA within a week under high humidity and temperature conditions (Champagne and Hron, 1992).

Untreated rice bran samples in this study had higher FFA increase than microwave-treated rice bran. The highest differences were among raw rice bran packed in vacuum pack and Ziplock® bags stored at room temperature over 16 weeks of storage (Figure 4). This difference has been shown by other researchers (Sharp and Timme, 1986). These investigators used polyethylene bags, metal cans, and vacuum sealing for metal cans as packaging methods and stored the samples at 3°C, 22°C and

38°C for 9 months. They performed chemical and sensory analysis and noticed the difference in FFA content and conjugated diene hydroperoxide in vacuum packing (higher amount) and regular sealed polyethylene bags. They concluded that the combination of 3°C storage temperature and sealed polyethylene provided the best shelf-life for brown rice.

The results of analysis of variance showed that all main effects and interactions were significant at the 0.05 level and insignificant for interaction of treatment, packaging, storage temperature, and weeks of storage. Extended storage resulted in an increase in FFA values. As a main effect, the packaging methods showed a difference in FFA between vacuum packed (more increase of FFA) and Ziplock® bags. Interaction between storage time and storage temperature showed that FFA content increased as storage time progressed (Figures 4 and 5). Untreated rice bran samples showed a higher increase in FFA than microwave stabilized rice bran. The lowest % FFA was in treated samples stored in the refrigerator. Interaction between storage time and packaging method showed a steady increase in FFA content as storage time increased, with the highest increase in vacuum packed samples.

Conclusions. The FFA values obtained in this study showed that microwave heat can be used as a method for inactivation of lipase to extend the shelf-life of rice bran. Storage temperature of 4-5°C worked better during 16 weeks of storage period compare to 25°C. The vacuum packing did not show any significant benefit than the non-vacuum packing. Therefore, it was concluded that, based on the conditions employed in this study, for prevention of hydrolytic rancidity in rice bran, the best

conditions would be microwave heat stabilized samples, Ziplock® bag packing, and stored in the refrigerator (4-5°C).

CHAPTER 4
PREVENTION OF OXIDATIVE RANCIDITY IN RICE BRAN DURING STORAGE

Introduction. Oxidative rancidity involves a reaction between the lipid and molecular oxygen. The reaction takes place at the double bonds of unsaturated fatty acids and can be accelerated by singlet oxygen, free radicals, metal ions (iron, copper, and cobalt), light, radiation, and enzymes containing a transition metal prosthetic group such as LOX (Barnes and Galliard, 1991). Rice bran contains minerals such as iron (130-530μg/g), aluminum (54-369μg/g), calcium (250-1310μg/g), chlorine (510-970μg/g), sodium (180-290μg/g), potassium (13,200-22,700μg/g), magnesium (9,860-12,300μg/g), manganese (110-880 μg/g), phosphorus (14,800-28,700μg/g), silicon (1700-7600 μg/g), and zinc (50-160 μg/g). Bran contains 80% of rice kernel iron (Lu and Luh, 1991). Also, the reactions involved are dependent on fatty acid composition (Nawar, 1985). LOX is found in a variety of plants, particularly the legumes, such as soybeans, mungbeans, navy beans, green beans, peas and peanuts, and in cereal such as rye, wheat, oat, barley and corn (Tappel, 1963). Unlike lipase, and like most other enzymes, LOX activity is accelerated by addition of water to cereal products (Barnes and Galliard, 1991).

LOX specifically oxygenates polyunsaturated fatty acids and/or their esters and acylglycerols containing the cis, cis-1,4 pentadiene double bound system located between carbons 6-10 counting from the methyl terminus (Shastry and Raghavendra Rao, 1975). Under appropriate conditions LOX leads to deterioration of fat-soluble vitamins and essential fatty acids (linoleic and linolenic acid) of oils and fats. It also

causes off-flavors and off-odors in food because of its reaction with unsaturated fatty acids. LOX-produced off-flavors are a significant potential problem in soybean products (O'Connor and O'Brien, 1991). There are different methods to inactivate lipoxygenase such as heating, pH adjustment and addition of antioxident (O'Connor and O'Brien, 1991). Brown et al (1982) succeeded in inactivation 99% of the LOX activity while retaining over 70% protein solubility by adjusting soybean moisture to 16.3% with pH 9.8 buffer and then heating with steam for 10 seconds. The effect of microwave heating on LOX stability has been investigated (Esaka et al., 1986, 1987; Wang and Toledo, 1987). Wang and Toledo (1987) demonstrated that the duration of microwave heating for LOX activity in soybean is shorter and the retention of protein solubility is higher than conventional heat processing. Esaka et al (1987) reported that LOX was completely inactivated in winged beans by microwave heating for 3 min with a 500 Watts power at 2450 MHz. Soaking the seeds before microwave heat treatment decreased the heating time needed to inactivate the enzyme. Various method of storage condition has been studied for brown rice to determine what temperature and type of packaging extend the shelf-life (Sowbhagya and Bhattacharya, 1976; Sharp and Timme 1986). Brown rice stored under modified atmospheres such as carbon dioxide flushing and under vacuum have shown to undergo slow changes in odor and flavor (Sharp and Timme 1986). different packaging methods can effect the storage stability of rice bran. A polyethylene and nylon combination film was found to be most suitable for long term storage of milled rice both under vacuum and with carbon dioxide flushing (Tai et al., 1981).

Little research has been done on the kinetics of LOX-catalyzed reactions in rice bran because of the complicated nature of the system. Although previous studies (Tao, 1989; Malekian, 1992) have shown that microwave processing of rice bran results in inactivating lipase responsible for hydrolytic rancidity, no information is available in the published literature as to how the deleterious effect of LOX activity on bran can be controlled during storage. Therefore, this study was undertaken to examine the effect of microwave heat on LOX activity in rice bran samples packed in Ziplock® bags and vacuum packed and stored at room temperature (25°C) or refrigerated (4-5°C) during 16 weeks of storage. The specific objectives were to: 1) optimize the pH for lipoxygenase enzyme extracted from rice bran samples, 2) optimize the temperature at which the rice bran LOX has the maximum activity, 3) determine the Michaelis costant (K_m) and maximum velocity (V_{max}) of lipoxygenase extracted from rice bran, 4) study the effect of microwave heat on rice bran LOX activity, 5) study the effect of packaging methods (vacuum packing vs. polyethylene Ziplock® bags) on rice bran LOX activity during 16 weeks of storage, 6) study the effect of temperature (25°C vs. 4-5°C) on rice bran LOX activity during 16 weeks of storage, 7) study the effect of microwave heat, two types of packaging methods, and storage time and temperature (25°C vs.4-5°C) on specific activity of LOX.

Materials and Methods. Material and methods are as follows:

Rice Bran Collection. Rice variety 'Lemont' (long grain), cultivated at the Louisiana Rice Experiment Station, Crowley, Louisiana was used for this experiment. The rice samples were dehusked and milled (friction type) by a Satake milling system

(Satake USA Houston, TX) at the Biological and Agricultural Engineering Department at Louisiana State University. Rice bran was collected in a barrel lined with a black plastic bag. Dry ice was added continuously to rice bran in the barrel during milling to prevent the hydrolysis of fatty acids by lipase activity. The bag was tied tightly and was delivered to the PBRC laboratory. The bags were placed in the ultra freezer (-78 to -80 C°) until the day of sample preparation (within 10 days). On the day of sample preparation, the samples were sieved with a 20 mesh siever in order to remove broken pieces of rice and husks. A thermometer was placed in rice bran samples to monitor the temperature (0-2°C) during the sieving process.

Microwave Heat Stabilization. One hundred fifty g per batch of raw rice bran were stabilized with a microwave oven, Sharp Carousel (Model R3A96, Sharp Electronic Corporation, NJ). The microwave chamber was heated before the stabilizing process by running the microwave at high for 3 minutes. The moisture content of raw rice bran samples, based on wet bases, was adjusted from initial 7.0 % to 21 % by using the calculation below:

$$150g \times 0.07 = 10.5ml$$

$$10.5\ ml + X = 0.21(150g + X)$$

$$10.5\ ml + X = 31.5g + 0.21\ X$$

$$X = 26.58\ ml$$

Therefore, 26.58 ml of deionized (DI) water were added to 150 g of rice bran sample to obtain 21% moisture content; the percentage of moisture content has been optimized in previous studies (Tao, 1989; Malekian, 1992). The sample was mixed

thoroughly with a stainless steel spatula to make sure that the water was evenly distributed. Each sample was placed in a Ziplock® one gallon storage bag (Hefty One Zip multi purpose storage bag Gallon size, Mobile Chemical Co., NY) spread out evenly to a thickness of 0.5cm and the bag was sealed until the end of microwave heating. The sample was heated at 100% power for 3 min. At the end of 3 min, the microwave door was opened and a hole was made in the bag so that a thermometer could be inserted into the sample. The temperature of the sample was 107 ± 2° C. The sample was cooled to 25°C. This was repeated until there was sufficient bran stabilized with microwave heat for the experiments. The samples were stored in an ultra freezer (-78 to -80°C) until the day of packaging (within 2 days).

Packaging and Storage of Rice Bran. Microwave heat stabilized and raw rice bran were divided in half, and from the first half, ten representative samples, each weighing 70-75g, were packed in polyethylene Ziplock® bags (Hefty One Zip multi purpose storage bag, Quart size, Mobile Chemical Co., NY), yielding a total of 20 bags. The bags were marked for storage time of 0, 4, 8, 12, and 16 weeks.

The other half were placed in an ice box, covered with dry ice, and delivered to the LSU Animal Science Department to be vacuum packed. The temperature of the room was 4-5° C. Ten representative samples, each weighing 70-75g, prepared from raw and microwave stabilized bran, were placed in non-permeable vacuum bags and vacuum packed. The vacuum machine used was TurboVAC (Model SB600, Howden Food Equipment, Netherlands). The bags were marked for storage time of 0, 4, 8, 12, or 16 weeks. The samples were brought back to the PBRC laboratory in the ice box .

All bags were also marked for FFA, fatty acid composition, LOX activity, and proximate analysis (at 0 and 16 weeks of storage). Half of the bags (20 bags total, 5 of each treatment) were stored at 4-5°C and the other half (20 bags) at room temperature (25°C). Room temperature and the temperature in the refrigerator were monitored and recorded daily. Figure 15 shows the schematic of rice bran processing and storage. Approximately 2,450g rice bran was used for this experiment.

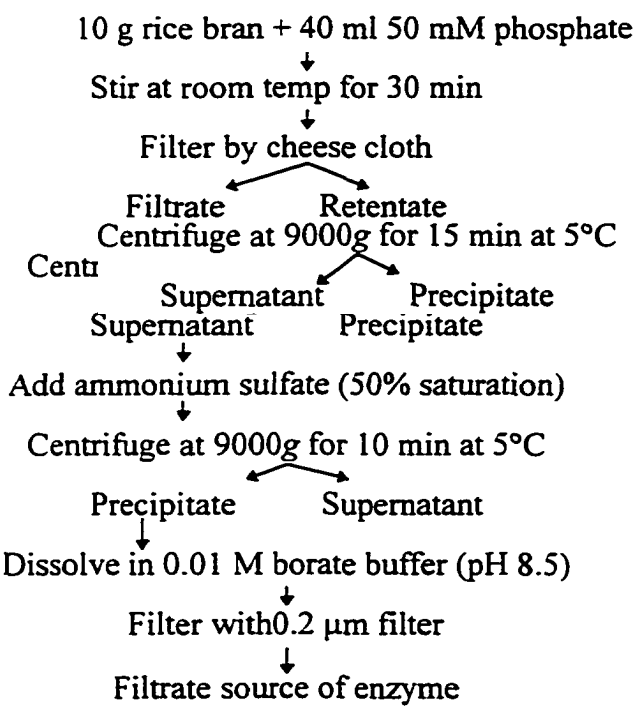
Lipoxygenase Activity Determination in Rice Bran. LOX activity was determined using the methods as described by Dixon and Webb (1961), Shastry and Raghavendra Rao (1975), and Aurand et al. (1987), with modifications. The modified method used in this study is described below.

Standard Enzyme Preparation. Soybean lipoxygenase (# L 7395 lot # 118 F03422) was purchased from Sigma Chemical Co., MO. This standard enzyme contained 110,600 units per mg solid. An enzyme solution was made by adding 11.6 ml of 0.01 M borate buffer, pH 8.5 (Corning pH meter # 340, Corning Inc., NY) to 1mg dried standard enzyme to obtain 10,000 units of enzyme per ml of buffer. The enzyme standard solution was used for each analysis as a control.

Enzyme Extraction from Rice Bran Samples. In a beaker, 10 g of rice bran sample was well mixed with 40 ml of 50 mM phosphate buffer (dibasic sodium phosphate, anhydrous (# 3827-01, J.T. Baker Inc., NJ) pH 7.0 for 30 min at room temperature. The sample was filtered into another beaker using two layers of cheese cloth. The filtrate was collected in a 50 ml (114 X 29 mm) graduated plastic conical tube (# 62.547.004. Sarstedt inc., NC) and was centrifuged (Model J2- HC, Beckman

Instrument Co., TX) at 9000*g* for 15 min at 5°C. The supernatant was collected in another 50 ml graduated plastic conical tube. The volume of the supernatant was recorded. Solid ammonium sulfate (A-2939, Sigma Chemical Co., MO) was added to each sample to obtain 50% saturation (Copper, 1942). The sample was mixed gently and centrifuged at 9000*g* for 10 min at 5°C. The volume was recorded. The supernatant was discarded and the precipitate was dissolved in .01 M borate buffer (sodium borate S-9640, Sigma Chemical Co., MO) pH 8.5, and the volume was adjusted until the previously recorded volume was obtained for each sample. This solution was filtered with a UNIFLO10 cc disposable syringe (# 309604, Becton Dickinson & Co., NJ) and filters (.2 μm pore size, 25 mm in diameter non-sterile units, Schleicher & Schuell., NH), and the filtrate was used as the source of enzyme. For assay, the enzyme extract was diluted 1 to 1 with .01 mM borate buffer pH 8.5.

The flow diagram of the LOX extraction follows:



Substrate Preparation. To a 100 ml volumetric flask, 100 µl of linoleic acid (# U-50-A, with purity greater than 99%+, NuChek Prep, Inc., MN) and 60 ml of absolute alcohol (200 proof, McCormick Distilling Co., Inc., MO) was added. The mixture was mixed gently into an emulsion and then, with slow stirring, then DI water was added to bring the volume to 100 ml. This was used as a stock solution. For assay,1 ml of stock solution was diluted with 6 ml of 0.01 M borate buffer, pH 8.5 (flow diagram below). The concentration of linoleic acid with 1 to 6 dilution was 0.4571 mM per ml. The flow diagram of substrate preparation follows:

100 µl of linoleic acid

↓

Dissolve in 60 ml absolute alcohol (in 100 ml volumetric flask)

↓

Volume to 100 ml with DI water

↓

Stock solution of substrate

↓

1 ml of stock solution + 6 ml of 0.01 M borate buffer (pH 8.5)

To study lipooxygenase activity extracted from rice bran, the optimum pH, optimum temperature, K_m (Michaelis constant), and V_{max} (maximum Velocity) was determined.

Optimum pH for Lipoxygenase Activity. Substrate solutions were made using the substrate stock solution and 0.01M borate buffer. The pH of the solutions were adjusted to 5, 6, 7, 8, 8.5, 9, and 10 with HCL and NaOH. Enzyme activity was measured with a thermostated spectrophotometer (Model # Du 640 Beckman Instrument, Inc., TX). The time was set for 5 min, the temperature at 25°C (ambient temperature), and the absorbency at 234_{nm}. The cuvette with 2.9 ml of substrate

solution was placed in the cell of the spectrophotometer. The enzyme solution (0.1 ml) was added as rapidly as possible, mixed well, and the increase in absorbency (A) at 234_{nm} versus the blank was recorded. One unit of LOX activity is defined as the change in absorbency of 0.001/min in 3 ml volume and 1 cm light path when linoleic acid is used as substrate (Shastry and Raghavendra Rao, 1975).

Optimum Temperature for Lipoxygenase Activity. Rice bran LOX activity was measured at 15, 20, 25, 30, 40, and 50°C by adjusting the thermostat in the spectrophotometer (Model # Du 640 Beckman Instrument, Inc., TX). The time was set for 5 min and the pH of the substrate solution was 8.5. The cuvette with 2.9 ml of substrate solution was placed in the cell of the spectrophotometer. The enzyme solution (0.1 ml) was added as rapidly as possible, mixed well, and the increase in absorbency (A) at 234_{nm} versus the blank was recorded. One unit of LOX activity is defined as the change in absorbency of 0.001/min in 3 ml volume and 1 cm light path when linoleic acid is used as substrate (Shastry and Raghavendra Rao, 1975).

Lipoxygenase Kinetics. Michaelis constant (K_m) and maximum Velocity (V_{max}) were determined in duplicate using linoleic acid stock solution diluted with 0.01 M borate buffer pH 8.5 to attain 4 different concentrations (0.1231, 0.0627, 0.0421, and 0.0317 mM). The reaction was followed in the spectrophotometer (Model Du 640, Beckman Instrument Inc., TX) at 25°C. The cuvette with 2.9 ml of substrate solution was placed in the cell of the spectrophotometer. The enzyme solution (0.1 ml) was added as rapidly as possible, mixed well, and the increase in absorbency (A) at 234_{nm} versus the blank was recorded and data were plotted according to Lineweaver

and Burk (1934). One unit of LOX activity is defined as the change in absorbency of 0.001/min in 3 ml volume and 1 cm light path when linoleic acid is used as substrate (Shastry and Raghavendra Rao, 1975).

Procedure for Determination of Lipoxygenase Activity. Enzyme activity was measured with a thermostated spectrophotometer (Model # Du 640 Beckman Instrument, Inc., TX). The time was set for 5 min, the temperature at 25°C (ambient temperature), and the absorbency at 234_{nm}. Into two Quartz cuvettes, in 3 ml volume (1 cm light path), labeled "blank" and "sample ID", the following was pipetted :

	blank	sample
substrate solution	3.0ml	2.9ml
enzyme solution	0.0ml	0.1ml

The cuvette with 2.9 ml of substrate solution was placed in the cell of the spectrophotometer. The enzyme solution was added as rapidly as possible, mixed well, and the increase in absorbency (A) at 234_{nm} versus the blank was recorded. One unit of LOX activity is defined as the change in absorbency of 0.001/min in 3 ml volume and 1 cm light path when linoleic acid is used as substrate (Shastry and Raghavendra Rao, 1975). The rate of increase was usually highest between 0.1-3.0 min, after which it decreased. The maximum increase in absorbancy per min at 234_{nm} was noticed between 0.1-3.0 min which was used to determined the specific activity.

Protein Assay. Protein concentration of enzyme solution was determined using the Warburg-Christian method (Layne, 1957). The absorbency of each protein-containing solution was measured on a spectrophotometer (Model # Du 640, Beckman Instrument Inc., TX) at 280 and 260 nm in duplicate. The ratio of these two values

was determined and used to select the appropriate correction factor provided as a table (Cooper, 1942; Layne, 1957). The absorbency at 280 nm was multiplied by the factor to yield the protein concentration in milligrams per milliliter:

(A_{280nm})(correction factor) = mg/ml protein

Specific Activity of Lipoxygenase. From the observed changes in absorbency per minute (correcting blank was used) the enzyme activity per ml of enzyme extract was calculated (Shastry and Raghavendra Rao, 1975 and Aurand et al., 1987).

$$\text{Specific activity} = \frac{dA_{234nm}/\text{min}}{\text{mg/ml protein}}$$

Statistical Analysis. A completely randomized design was used. To study the main effect for each factor, three factor factorial (2x2x2) and four factor factorial (2x2x2x4) arrangements (Table 11 in Appendices) were used for each variable. To compare the mean of the results (Table 12 in Appendices), the Student-Newman-Keuls (SNK) test was done (generally this test declare more significant differences than other tests such as Tukey's procedure). A statistical analysis of variance (ANOVA) was performed on all values using the SAS® program version 6.12 (SAS, 1997). Differences were considered significant when means of compared sets differed at $p < 0.05$ level of significance.

Results and Discussion. The results obtained in this study are as follows:

Optimum pH for Lipoxygenase Activity. The pH profile of LOX activity is shown in Figure 6. The optimum activity was about 8-8.5 at room temperature (25°C). Shastry and Raghavendra Rao (1975) noticed optimum pH of 8.5 and a little

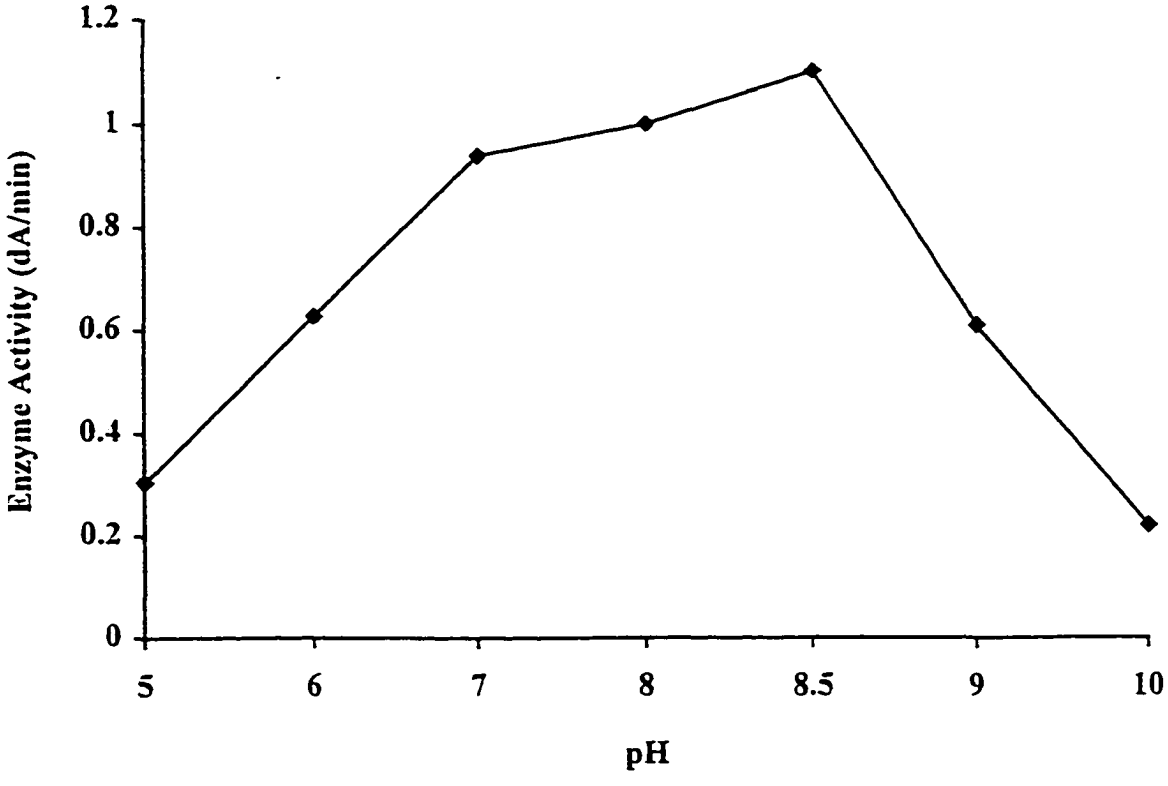


Figure 6. Optimum pH for lipoxygenase activity in rice bran at room temperature (25°C).

detectable activity at pH 6.5 in LOX extracted from fresh raw rice bran. Sekhar and Reddy (1982) performed a study on LOX from eight scented and two unscented rice varieties. They found that all the varieties with the exception of IR-8 had the optimal activity around pH 8. The variety IR-8 showed a different type of activity with maximum activity around pH 6. Yamamoto et al. (1980) showed an optimum pH of 6.5-7 for partially purified rice germ LOX. Ida et al. (1983) reported three distinct peaks of LOX activity in embryos of rice, L1, L2, and L3, each having a different pH optimum of 4.5, 5.5, and 7, respectively. The results obtained in this study are very similar to the data obtained by Shastry and Raghavendra Rao (1975) and Sekhar and Reddy (1982).

Optimum Temperature for Lipoxygenase Activity. The study of rice bran enzyme extract at different temperatures (15, 20, 25, 30, 40, and 50°C) using the 1 to 6 diluted substrate solution (from stock solution), revealed (Figure 7) that enzyme activity increased with increased in reaction temperature from 15°C until it reached 25°C and then started decreasing sharply at temperature greater than 30°C. The temperature of 25°-30°C was optimum at pH 8.5. Room temperature has been used for studying LOX activity by a number of researchers (Surrey, 1964; Wallace and Wheeler, 1972; Shastry and Raghavendra Rao, 1975; Sekhar and Reddy, 1982).

Lipoxygenase Kinetics. Enzyme activity was measured at different concentrations of the linoleic acid substrate (0.1231, 0.0627, 0.0421, and 0.0317 mM). The results are graphically presented using the Lineweaver-Burk plot (Figure 8). The K_m value was 0.097 mM and V_{max} of 0.834 dA/min with linoleic acid as substrate, pH

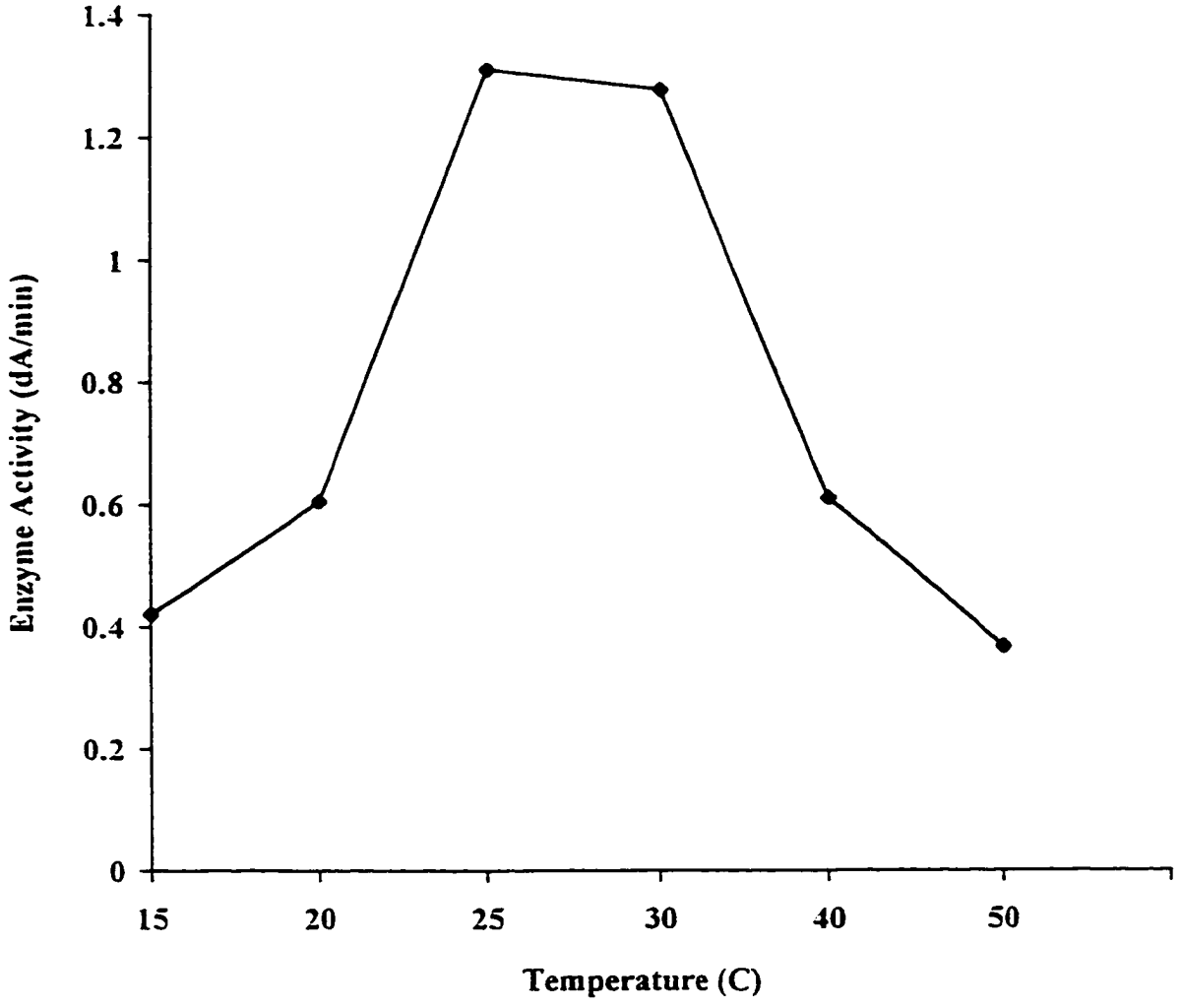


Figure 7. Optimum temperature for lipoxygenase activity in rice bran at pH 8.5.

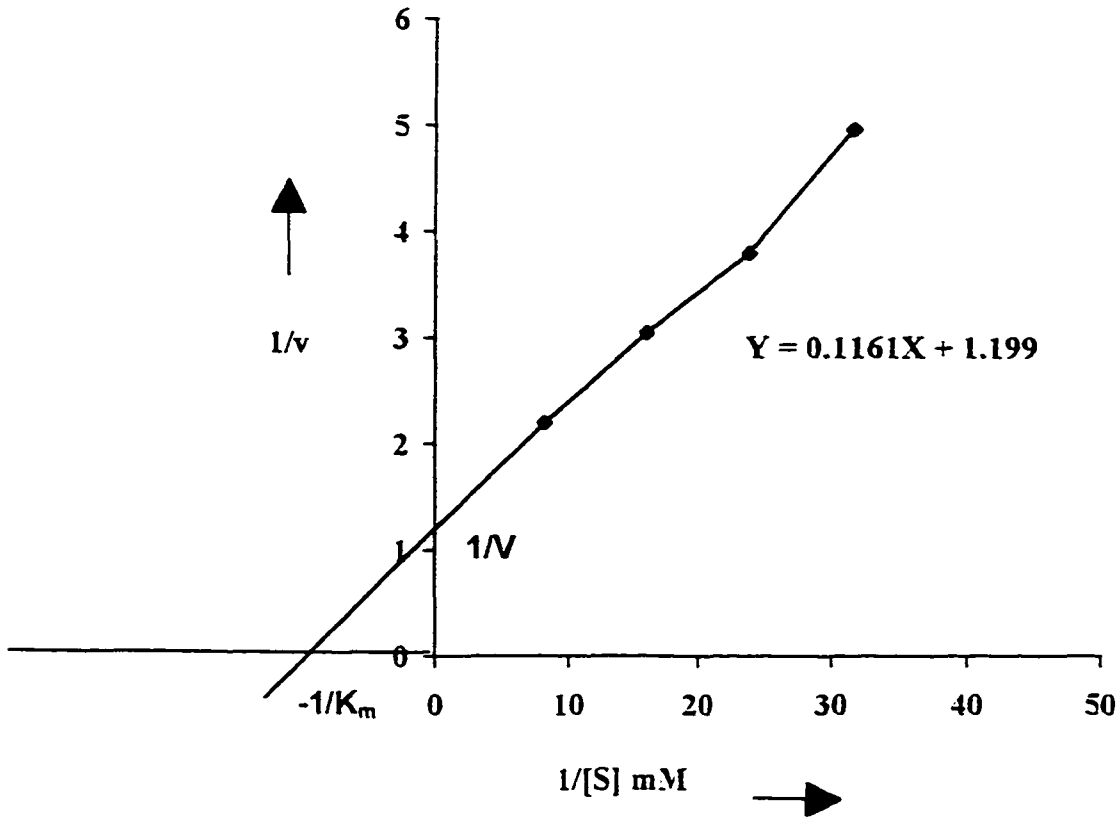


Figure 8. Determination of K_m according to Lineweaver and Burk for LOX activity in rice bran. Activity was measured spectrophotometrically. K_m= Michaelis constant, S= substrate, v= velocity, V= maximum velocity.

of 8.5, and 25°C. These two parameters (K_m and V_{max}) can be used to give a quantitative measure of the specificity of an enzyme for a given substrate and the efficiency of an enzyme. The higher the ratio of V_{max}/K_m, the greater the preference (or specificity) of the enzyme towards that substrate (Price and Stevens 1991). Yamamoto et al. (1980) concluded that the rice germ enzyme reaction was highly specific for linoleic and linolenic acids, with a K_m value of 0.1 mM at pH 7 and 25°C. The result of this study is therefore in agreement with that of Yamamoto et al. (1980). They suggested that initial velocity of the reaction was proportional to the enzyme concentration, and the linear relationship was characteristic of the LOX-catalyzed reactions. Shastry and Raghavendra Rao (1975) reported a Km value of 0.35 mM for LOX extracted from rice bran with linoleic acid as substrate and pH 8.5 and 25°C. They suggested that this value was tentative since a technical grade linoleic acid was used, and the enzyme was not homogeneous. The K_m values of 1 and 5mM have been reported for soybean and wheat, respectively and K_m values of 0.3, and 0.8 for potato has been reported (Shastry and Raghavendra Rao 1975). Linoleic acid dispersed in TritonX-100 and Brij had K_m values of 0.175mM and 0.157 mM and V_{max} values of 0.225 and 0.532 dA/minute, respectively (Boyes et al., 1992). The reaction was carried at pH 7.0. Macri et al. (1994) reported a K_m value of 0.2 mM at pH 5.5-6.0 for both linoleic and linolenic acids in highly purified soybean plasma membranes. The plasma membrane also showed a LOX activity in the alkaline (pH 9.0-9.5) range with a lower K_m value of 0.06 mM. K_m (Michaelis constant) is by no means an absolute constant, but depends on pH, temperature, effectors, buffer, etc. (Michal, 1978).

Effect of Microwave Heat on Lipoxygenase Activity. Results obtained from effect of microwave heat, packaging methods, and storage temperature during 16 weeks of storage on LOX activity and specific activity were analyzed and discussed. Figure 9 shows that LOX activity increased slightly at week 4 in raw rice bran in Ziplock® bags and stored at room temperature, while samples stored in the refrigerator had a slight decrease (Figure 10). Raw rice bran samples vacuum packed and stored up to 4 weeks in the refrigerator did not show significant changes (Figure 10), while the samples stored at room temperature had a slight increase (Figure 9). These changes were not significant. The main effects and interaction were not significant ($p< 0.05$) except for the main effect of room temperature. Extended storage at room temperature resulted in fluctuating LOX activity (Figure 9). At 8 weeks of storage LOX activity fluctuated but these changes were not significant for samples kept in refrigerator. The main effects and interactions were not significant ($p< 0.05$) for all factors except for the main effect of storage temperature. After 12 weeks of storage the LOX activity of microwave heated samples was drastically increased, but that of raw samples was drastically decreased, regardless of the packing types (Figure 9). The main effect of storage temperature was highly significant ($p < 0.001$) and interaction of storage temperature and packaging methods was significant ($p < 0.05$). The raw rice bran samples stored for 16 weeks had a significant ($p < 0.05$) decrease in LOX activity (in both packaging methods) compared to 0 weeks (Figure 9). The main effects of treatment and storage temperature were significant ($p < 0.05$). The interaction of treatment and storage temperature was highly significant.

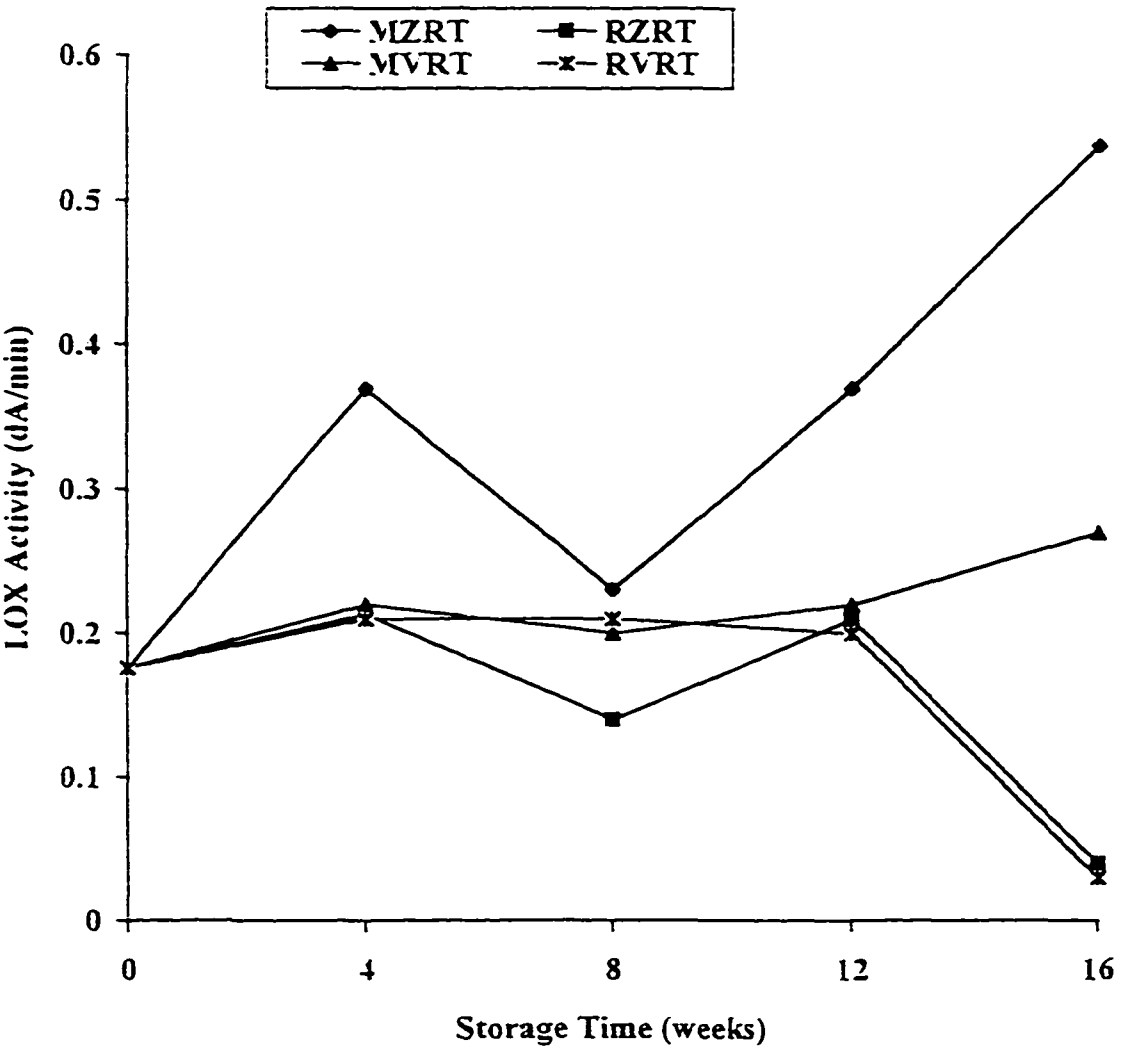


Figure 9 Lipoxygenase activity in raw (control) and microwave heat stabilized rice bran packed in Ziplock® bags and vacuum packed and stored at room temperature (25°C) Individual values, means and standard deviations presented in Table 15 in the Appendices. R= raw, M= microwave heat stabilized, Z= Ziplock® bags, V= vacuum packed, RT= room temperature

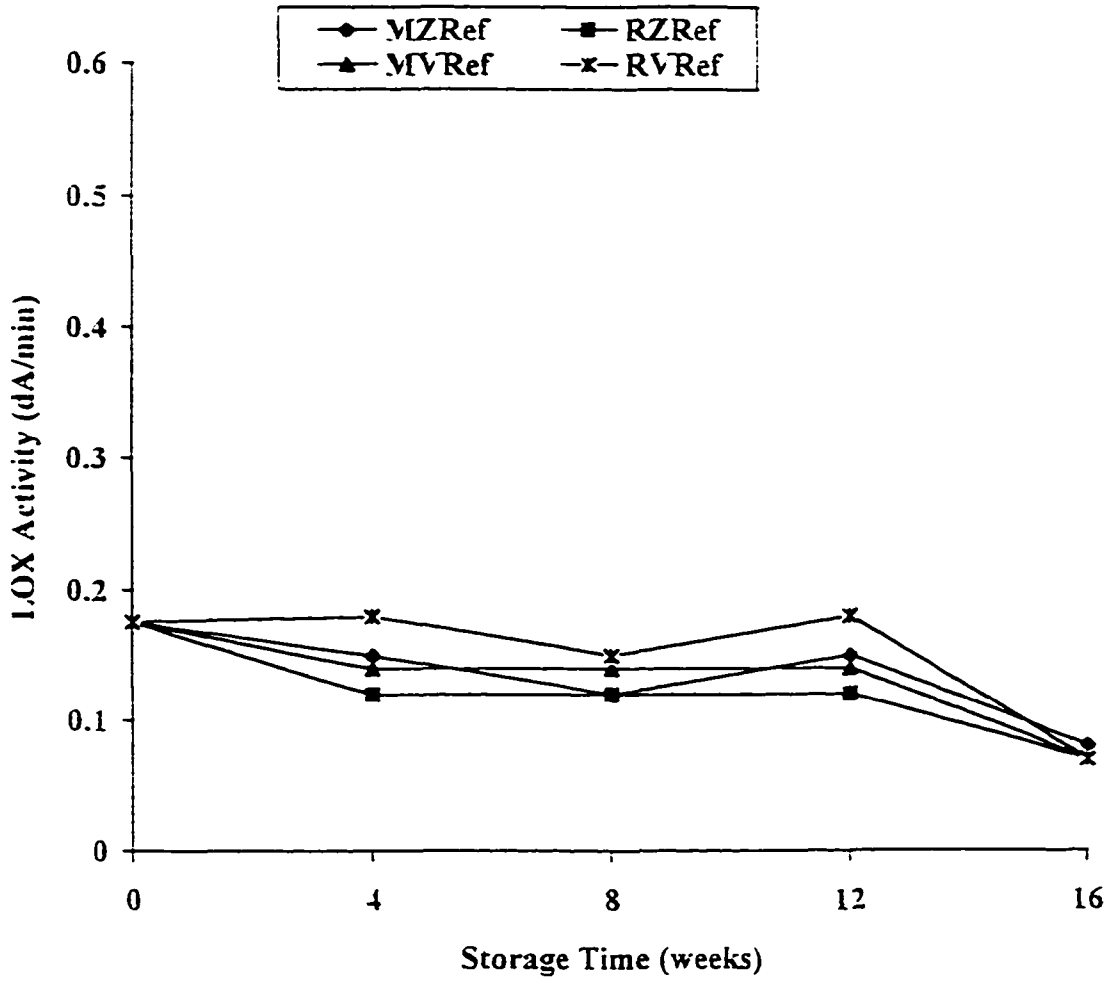


Figure 10. Lipoxygenase activity in raw (control) and microwave heat stabilized rice bran packed in Ziplock® bags and vacuum packed and stored in the refrigerator (4-5°C). Individual values, means and standard deviations presented in Table 16 in the Appendices. R= raw, M= microwave heat stabilized, Z= Ziplock® bags, V= vacuum packed, Ref= refrigerator

The microwave heat stabilized rice bran kept refrigerated did not show (Figure 10) any significant changes at week 4 of storage. There was a significant ($p < 0.05$) difference in the LOX activity between samples stored at room temperature and samples stored in the refrigerator (Figure 9 verses Figure 10). During week 12 and 16, there were no significant changes in LOX activity for microwave heated samples, except for a significant ($p < 0.05$) increase of LOX activity in samples packed in Ziplock® bags and stored at room temperature (Figure 9).

Interaction between storage temperature and treatment showed (Figure 9) a difference in LOX activity levels between microwave heated samples packed in the Ziplock® bags. The samples were stored at room temperature and in the refrigerator. LOX activity increased for samples stored at room temperature while there was not much change in samples stored in the refrigerator. Microwave heat stabilized samples showed fluctuation with a peak at 4 weeks, then a decrease at 8 weeks, and a sharp increase throughout the rest of the storage period (Figure 9). This fluctuation has been demonstrated by Sharp and Timme (1986). They used long grain (Starbonnet) brown rice and packed it in sealed polyethylene bags, sealed bags in metal cans, and punctured sealed bags sealed in a metal can under vacuum. They stored the samples in three different temperatures (3°C, 25°C, and 38°C) for 9 months. LOX activity was expressed as a concentration of conjugated diene hydroperoxides (CDHP). They showed that CDHP increased between month 1 and 2 and decreased between months 2 and 3, regardless of storage temperature, which is in agreement with data obtained in this study.

The LOX activity in rice that was microwave heat stabilized and stored at room temperature did not increase as sharply as samples packed in Ziplock® bags (Figure 9). This could be due to lack of oxygen, the co-substrate (Berry et al., 1997), which was taken out during vacuum packaging. LOX catalyzes the oxidation of methylene-interrupted unsaturated fatty acids and their esters such as linoleic and linolenic acid (Richardson and Hyslop, 1985). The native-resting enzyme, a relatively inactive form, is high-spin ferrous, which is converted to the active ferric form by oxidation, possibly with atmospheric oxygen (Gardner, 1988).

The interaction between treatment and packaging method showed that raw samples packed in Ziplock® bags exhibited a sharp decrease in LOX activity after 12 weeks of storage (figure 9). Similar pattern is shown for vacuum packed samples. The rice bran sample stored in the refrigerator showed no significant ($p < 0.05$) change up to 12 weeks and a decrease at the end of storage period.

The results of this study showed that, overall, the samples stored at room temperature had higher LOX activity than samples stored in the refrigerator. increase in CDHP level from increased in temperatures have been previously reported (Mitsuda et al., 1972; Sowbhagya and Bhattacharya, 1976). This could be due to the effect of light and storage temperature. The striking effect of light has been shown (Sowbhagya and Bhattacharya, 1976) in lightly milled, cured, and parboiled rice lipid during storage at room temperature. Oxidative rancidity was much higher in samples placed under light all the time compare to the same samples kept in containers and placed in the dark. The light caused an increase in oxidation rancidity in samples stored at room

temperature. Sowbhagya and Bhattacharya (1976) concluded that a relatively high moisture content, storage in the dark, and low temperatures, were the important protectants of rice lipids against rancidification.

Microwave heat stabilized samples packed in Ziplock® bags and stored at room temperature had much higher LOX activity than the raw rice bran kept under the same conditions. This could be due to the lack or loss of antioxidants present in rice bran samples during microwave heating. As early as 1943, Gyorgy and Tomarelli noticed that brown rice had beneficial antioxidant activity which was reduced either upon milling or upon autoclaving (120°C, 30 min). Rice bran and rice bran oil contain a good amount of potent antioxidant such as oryzanol, ferulic acid, and esters of unsaturated triterpenoid alcohols (Sowbhagya and Bhattacharya, 1976). These compounds can be lost at the time of milling (Sowbhagya and Bhattacharya, 1976) and/or lose their activity or be destroyed during microwave heating (Yoshida et al., 1991). Microwave heating for various periods of time destroyed α-tocopherol. The loss of activity in tocopherols was found to increase in the order δ-, β-, γ-, and α-tocopherol (the most potent) with microwave heating of the oils. The effectiveness of tocopherols as lipid antioxidants has been attributed to their ability to break chain reactions by reacting with fatty acid peroxy radicals. The findings in this study agree with the above conclusions. As a result, the LOX activity increased. Also, the increase in LOX activity in microwave heat stabilized rice bran stored at room temperature observed in this study could be due to an increase in the concentration of transition metals such as copper, cobalt, chromium, and especially iron. This was

demonstrated by the study of Rao and Artz (1989) who applied extrusion temperature to a corn starch/soybean oil mixture. They reported that most of metals, especially iron, were present at concentrations that were highly catalytic with respect to oxidation (LOX activity). An increase in iron concentration for the the samples extruded at 135 and 175°C was nearly 3 and 6 times that of the unextruded samples, respectively (Rao and Artz, 1989). This iron was considered to contribute to higher lipid instability in extruded samples in higher temperature. Also, Rao and Artz (1989) noted that some of the increase in oxidation may be due to the increase in surface area associated with an increase in expansion ratio. Shastry and Raghavendra Rao (1975) reported that partially purified LOX from unfractionated rice bran of an indica variety was activated by Fe^{++}. Hiroyuki et al. (1986) indicated that rice LOX-3 was inactivated gradually in storage because of participation of metal ions and linoleic hydroperoxide. Champagne et al. (1992) reported that increased susceptibility of ethanol-extracted brown rice kernel to oxidative deterioration could be attributed to ethanolic and heat denaturation of the homoproteins catalase and peroxidase found in the kernel. They suggested unfolding the enzymes to bring about greater exposure of the heme groups to the substrate, allowing the heme group to initiate oxidation. Microwave heat employed in this study (107 ± 2°C, for 3 min) could be a contributing factor to increase in LOX activity in microwave heat stabilized samples compared to untreated samples.

Increase in LOX activity during storage has been demonstrated by Dhaliwal et al. (1991). They concluded that drying the rice paddy before storage did not affect LOX activity in milled rice, but activity increased significantly while samples were

stored at room temperature for 12 months. More increase was noticed for long rice grains (Basmati-370) compared to short (IR-8) and medium (PR-108) grain varieties. Since LOX acts on unsaturated fatty acids like linoleic acid, which comprises up to 40-45% of the fatty acids in rice, it can be assumed that the varieties with lower activities of this enzyme may have better storage qualities (Sekhar and Reddy, 1982).

The moisture content of microwave stabilized rice bran packed in Ziplock® bags and stored at room temperature at the end of the storage period was significantly ($p< 0.05$) lower than the samples at 0 weeks (Table 1). The vacuum packed microwave heat stabilized samples (VRT) as the samples packed in the Ziplock® bags (ZRef) had the same amount of moisture. Although the moisture content was nearly equal (6.3-6.4%), the microwave heat stabilized bran kept refrigerated (ZRef) had significant lower LOX activity than those kept at room temperature, regardless of the packaging types (ZRT and VRT). These samples had been dehydrated, which may have led to lower water activity in the rice bran samples. The moisture content of rice after drying in an open Petri dish for 24 hours was 2-3% which caused the increase in oxidation of lipids (Sowbhagya and Bhattacharya, 1976). A decrease in water activity accelerates the oxidation of lipids in food systems (Koch, 1961; Lee, 1975). Microwave heat stabilized samples stored in the refrigerator regardless of packaging had a steady and significant decrease in LOX activity during 16 weeks of storage.

Raw rice bran samples (control) did not show significant ($p > 0.05$) change in LOX activity except for raw samples packed in Ziplock® bags and stored at room temperature. There was a significant decrease ($p < 0.05$) after 8 weeks, an increase at

Table 1. Lipoxygenase (LOX) activity, free fatty acid (FFA), and moisture content of rice bran packed in Ziplock® bags (Z) or vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

			LOX Activity (dA/min)	FFA (%)	Moisture (%)
Raw(Control)	0 week		0.18 ± 0.00^{b}	2.53 ± 0.2^{f}	7.5 ± 0.1^{b}
	16 weeks	ZRT	0.04 ± 0.01^{e}	48.01 ± 1.0^{b}	7.0 ± 0.0^{bc}
		ZRef	0.07 ± 0.00^{e}	19.45 ± 1.8^{d}	9.3 ± 0.1^{a}
		VRT	0.03 ± 0.00^{e}	54.29 ± 0.6^{a}	6.5 ± 0.2^{c}
		VRef	0.07 ± 0.02^{e}	25.37 ± 0.3^{c}	8.5 ± 0.2^{a}
Microwave	0 week		0.18 ± 0.00^{b}	2.75 ± 0.1^{f}	8.4 ± 0.4^{a}
	16 weeks	ZRT	0.54 ± 0.11^{a}	10.93 ± 0.6^{e}	6.4 ± 0.2^{c}
		ZRef	0.08 ± 0.01^{e}	3.58 ± 0.1^{f}	6.3 ± 0.1^{c}
		VRT	0.26 ± 0.10^{b}	11.62 ± 0.0^{e}	6.3 ± 0.6^{c}
		VRef	0.07 ± 0.03^{e}	3.74 ± 0.2^{f}	7.6 ± 0.7^{b}

[a]*Means (average of 2 values) within a column with different letters are significantly different* ($p \leq 0.05$).

week 12, and a highly significant decrease ($p < 0.001$) after 12 weeks of storage (Figure 9). Sowbhagya and Bhattacharya (1976) observed that even with rapid production of FFA in raw rice the oxidation remained so slow. They suggested that either the oxidation-retarding factors in raw rice were active or the specificity of the hydrolytic enzyme(s) and the pattern of release of the acids were such that did not favor oxidation. The slow LOX activity in raw rice bran samples also could be due to the action of 9(S)-hydroperoxyoctadecatienoic acid (9(S)-HPOT) derived from linoleic acid which inactivated the LOX. Dai-Eun and MeeRee (1989) reported that that incubation of α-linoleic acid with LOX initially at 4°C and subsequently at 25°C was required for maximal production of the conjugated triene acids, the condition required for the maximal inactivation of LOX activity. They reported that soybean LOX was inactivated gradually during the conversion of 9(S)-HPOT to 9,16-dihydroperoxy conjugated triene acid; therefore, α-linoleic acid or 9(S)-HPOT was used as a substrate to form an unstable peroxide which could inhibit the soybean LOX irreversibly, following a suicide substrate mechanism (Williamss et al., 1986). LOX-3 is the major enzyme in rice grain (Hiroyuki et al., 1986) which specifically produces 9(S)-HPOT (Ida et al., 1983). The reduction in LOX activity while the substrate concentration was increased in the absence of calcium ion, showing a maximum of 0.2mM of linoleate, could be due to substrate inhibition, which is often attributed to the choking of the catalytic site by two molecules of substrate (Yamamoto et al., 1970). This information supports data obtained in this study: the reduction in LOX activity in untreated samples (increase in the amount of substrate by the action of

lipase and presence of antioxident) and increase in microwave heat stabilized samples (lack of substrate ,more exposure of metal ions such as iron and calcium, and lack of antioxidents).

Specific Activity of Lipoxygenase. The specific activity, expressed as dA/min/mg of sample, of microwave heat stabilized and untreated rice bran is shown in Table 2. The changes in specific activity are not significant at 4 weeks of storage. After 8 weeks there was a drastic increase in microwave heat stabilized samples vacuum packed and stored at room temperature. At week 12, microwave heat stabilized samples stored at room temperature in a Ziplock® bag showed a significant ($p < 0.05$) increase, and the rest of the samples decreased but not significant. At each storage time interval, the specific activity of the microwave heat stabilized bran kept refrigerated either in the Ziplock® bags or vacuum packed showed no significant difference. For the microwave heat stabilized bran, the specific activity of the sample in the Ziplock® bags kept at room temperature (ZRT) increased with increased storage time; the opposite effect was observed for the samples in the vacuum packed bags kept at refrigerated temperature (VRef). Data (Table 2) also showed that the specific activity of microwave heated bran in Ziplock® bag kept refrigerated (ZRef) drastically dropped after 8 weeks of storage as opposed to after 12 weeks of storage observed for VRef. However, the specific activities of both ZRef and VRef were similar (532.1-612.2 units/mg sample) (Table 2).

Hafez et al. (1985b) observed that there was a negative correlation between the dose of γ irradiation and the enzyme-specific activity in soybean. The highest

Table 2. Lipoxygenase (LOX) specific activity of rice bran packed in Ziplock® bags (Z) or vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
				Specific Activity units /mg sample		
Raw(Control)		1085.9±.14.2 [a]	1085.9 ±14.2[a]	1085.9 ±14.2[ab]	1085.9 ±14.2[b]	1085.9 ±14.2[b]
	ZRT		1464.4 ± 30.6[a]	1164.6 ± 40.5[ab]	816.0 ± 25.7[bc]	165.4 ± 9.6[d]
	ZRef		1001.1 ± 82.9[a]	943.6 ± 13.9[b]	464.0 ±11.9[c]	504.3 ± 26.3[c]
	VRT		1454.1 ± 11.9[a]	1676.1 ± 39.9[ab]	856.8 ± 16.5[bc]	170.5 ± 41.4[d]
	VRef		1153.1 ± 17.6[a]	870.7 ± 10.4[b]	787.2 ± 56.3[bc]	640.1 ± 92.6[c]
Microwave		1119.1±10.5 [a]	1119.1 ±10.5[a]	1119.1 ±10.5[ab]	1119.1 ±10.5[b]	1119.1 ±10.5[b]
	ZRT		1204.2 ± 10.8[a]	1259.5 ± 98.9[ab]	1535.3 ± 21.5[a]	2008.7 ±35.7[a]
	ZRef		928.5 ± 12.6[a]	1094.4 ± 88.0[ab]	579.9 ± 74.6[bc]	612.2 ± 68.0[c]
	VRT		1273.4 ± 32.7[a]	1754.6 ± 65.1[a]	836.0 ± 21.6[bc]	1271.4 ±55.9[b]
	VRef		932.5 ± 19.5[a]	917.8 ± 16.8[b]	852.2 ± 15.5[bc]	532.1 ± 10.7[c]

[a]Means (average of 2 values) within a column with different letters are significantly different ($p \leq 0.05$).

inactivation was at 65 KGy and moisture content of 30.47% (173.03 ± 3.23 dA/min/g of defatted sample). The moisture content in activation of LOX activity was important. Esaka et al. (1987) suggested that microwave heat was more effective in inactivating LOX in winged bean seeds with higher moisture (soaking the seeds in water for 15 hr required 30 sec of microwave heating) to completely inactivate LOX. They concluded that microwave heating inactivates LOX of winged bean seeds in much less time than conventional heating. The same conclusion was obtained for soybeans (Esaka et al., 1986). Wang and Toledo (1987) reported that LOX activity in microwave heated soybean samples, with higher moisture content (38.8, 47.0, and 56.8%), were completely inactivated. The soybean temperature was around 100°C. This confirms that not only the temperature of the sample is important for LOX inactivation, but the moisture content of the sample plays an important role, since it results in higher energy absorption.

In our study, the microwave heat did not inactivate LOX activity, and this could be mostly due to the moisture content of the samples. The moisture content in rice bran samples were adjusted from an initial 7.5 to 21% before the stabilization process. The temperature in rice bran samples reached 107 ± 2°C during microwave heat processing. The amount of moisture in excess of 21% resulted in the bran becoming too clumpy, and moisture less than 21% resulted in the bran being too dry and burned in some areas (Tao, 1989; Malekian, 1992). It appears the moisture content or duration of time was not enough to inactivate the LOX activity. On the other hand, lipase was inactivated under the same condition.

Conclusions. LOX oxidizes polyunsaturated fatty acids rapidly but its action is dependent on the earlier release of polyunsaturated fatty acids from triglycerides by the action of lipases. Thus, measurement of lipase activity provides a means of predicting the relative rates of deterioration of bran samples. The results of this study showed that Rice bran LOX had optimum pH of 8.5 and optimum temperature of 25-30°C. The K_m value was 0.097mM and the V_{max} value of 0.847 dA_{234}/min. The results also showed that even though microwave heating did not inactivate LOX enzyme, it did inactivate lipase, and therefore, there was a reduced amount of substrate present for LOX activity in microwave heat stabilized samples stored in the refrigerator (4-5°C). Vacuum packing method did not show any significant advantage over Ziplock® bags. Specific activity was decreased in microwave heat stabilized samples stored in refrigerator regardless of packaging methods. Also it was concluded that, for prevention of lipid degradation in rice bran, samples should be treated with microwave heat (to inactivate lipases), packed in Ziplock® bags (the common and most- used packing material) and stored in the refrigerator (4-5°C). The condition above provides for very low lipase activity which, if not inactivated, otherwise promotes hydrolytic rancidity and the least LOX activity which, if not inactivated, otherwise promotes oxidative rancidity in rice bran samples.

CHAPTER 5
NUTRIENT LOSSES IN RICE BRAN DURING STORAGE

Introduction. Rice bran is rich in nutrients with a protein content of 14-16%. The nutritional value of rice bran protein, high in lysine, one of the essential amino acids, is relatively high. The reported protein efficiency ratio (PER) is 1.6-1.9, compared to the value of 2.5 for casein (Saunders, 1990). Major carbohydrates in rice bran are hemicellulose (8.7-11.4%), cellulose (9-12.8%), starch and β-glucan (1%). Rice bran contains 15-23% oil. Three major fatty acids, palmitic acid (12-18%), oleic acid (40-50%) and linoleic acid (30-35%), make up 90% of total fatty acids. Crude rice bran oil contains 3-4% waxes and about 4% unsaponified lipids. Oryzanol and vitamin E, potent antioxidants, are present in rice bran (Saunders, 1985). Rice bran is also rich in B-complex vitamins. The mineral composition of rice bran depends on nutrient availability of the soil in which the crop is grown. Rice bran contains iron, aluminum, calcium, sodium, potassium, magnesium, manganese, phosphorus, silicon, and zinc. Bran contains 80% of rice kernel iron (Lu and Luh, 1991).

Fatty acids are important in a number of functions in the human body. Linoleic acid, with two double bonds, is one of the essential fatty acids that can not be manufactured by the body. Linoleic acid is found at high concentrations in vegetable oil and, to a smaller extent in meats. It is the basic structural element of fats in the body, and therefore, is one of the essential nutrients required by the body. Rice bran is a good source of linoleic acid (30-35% of total lipid). The deficiency of linoleic acid has never been found in humans eating a normal diet (Labuza, 1977). The signs of

deficiency are dermatitis (skin disorders) and metabolic disorders of cholesterol and fat transport. The amount of linoleic acid in a normal diet is about 2% of the total calories or from 5 to 6 g per day. Two to three tablespoons of soybean oil (over 50% of linoleic acid) will supply the needed amount (Labuza, 1977). With rice bran oil (30-35% linoleic acid) the required amount is about 3-4 tablespoons per day.

Mivrowave ovens are considered among the most energy-efficient types, and the most rapid method for heating food items (Yoshida rt al., 1991). Foods containing high moisture and fat readily absorb microwaves and are cooked or baked. Structural and chemical changes occurring in food components on processing may produce undesirable nutritional effects. Soybean treated with microwaves for 6 min, (optimal to prepare full-fat soy flour without burnt odor), retained ca. 90% of the vitamin E and had changes of lipids(Yoshida and Kajimoto, 1989). Irradiation of soybean seeds of 7.5% moisture using microwave heat for 9, 12, and 15 min had little effect on the phospholipid composition (Hafez et al., 1985b). Increasing gamma-irradiation doses from 0 to 65 KGy significantly ($p < 0.05$) decreased the phospholipid content of soybean seeds (Hafez et al., 1985b).Microwave baking of potato increased the cortical contents of total amino acids by 25 and free amino acids by 8% and had only slight effects on mineral composition (Klein and Mondy, 1981). Microwave processing had little effect on proximate composition of bread baked with microwave heat (Tsen, 1977) and lysine became less available nutritionally with conventional baking than with microwave baking for 8 min at 300 Watts. Microwave baking breads were lighter in colors which indicates less browning reaction and less nutritional values.

Storage of rice seeds, rice grain, and rice bran for extended periods leads to a number of degradative changes that cause reduction in percentage germination. Lipid peroxidation (by lipases and lipoxygenases) is thought to be a primary cause for seed deterioration (Ramarathnam et al., 1989). Use of heat treatment (Lin and Carter, 1973; Sayre et al., 1982), microwave heat (Hafez et al., 1985a; Tao et al., 1993), and γ irradiation (Hafez et al., 1985b; Ramarathnam et al., 1989) as a method for food preservation has been studied. There was no change in proximate composition of microwave heat stabilized rice bran packed in Ziplock® bags and vacuum packed, stored at 4-5°C for 8 weeks (Malekian, 1992). The objectives of this study were:

1. To study the effect of microwave heat on fatty acid and proximate composition of rice bran
2. To study effect of temperature (25°C vs. 4-5°C) on fatty acid and proximate composition of rice bran during 16 weeks of storage
3. To study the effect of packaging method (vacuum packing vs. Ziplock® bags) on fatty acid and proximate composition of rice bran during 16 weeks of storage.

Materials and Methods. Material and methods are as follows:

Rice Bran Collection. Rice variety 'Lemont' (long grain), cultivated at the Louisiana Rice Experiment Station, Crowley, Louisiana was used for this experiment. The rice samples were dehusked and milled (friction type) by a Satake milling system (Satake USA Houston, TX) at the Biological and Agricultural Engineering Department at Louisiana State. Rice bran was collected in a barrel lined with a black plastic bag. Dry ice was added continuously to the rice bran in the barrel during

milling to prevent the hydrolysis of fatty acids by lipase activity. The bag was tied tightly and was delivered (within 15 min) to the Pennington Biomedical Research Center (PBRC) laboratory. The bags were placed in the ultra freezer (-78 to -80 C°) until the day of sample preparation (within 10 days). On the day of sample preparation, the samples were sieved with a 20 mesh siever in order to remove broken pieces of rice and husks. A thermometer was placed in rice bran samples to monitor the temperature (0-2°C) during the sieving process.

Microwave Heat Stabilization. One hundred-fifty g per batch of raw rice bran were stabilized with a microwave oven, Sharp Carousel (Model R3A96, Sharp Electronic Corporation, NJ) 850 Watts power at 2450 MHz. The microwave chamber was heated before the stabilizing process by running the microwave at high for 3 min. The moisture content of raw rice bran samples was adjusted on wet basis from initial 7.0 % to 21 % by using the calculation below:

$$150g \times 0.07 = 10.5ml$$

$$10.5\ ml + X = 0.21(150g + X)$$

$$10.5\ ml + X = 31.5g + 0.21\ X$$

$$X = 26.58\ ml$$

Therefore, 26.58 ml of deionized (DI) water were added to 150 g of rice bran sample to obtain 21% moisture content; the percentage of moisture content has been optimized in previous studies (Tao, 1989; Malekian, 1992). The sample was mixed thoroughly with a stainless steel spatula to make sure that the water was evenly distributed. Each sample was placed in a Ziplock® one gallon storage bag (Hefty One

Zip multi purpose storage bag Gallon size, Mobile Chemical Co., NY)and spread out evenly to a thickness of 0.5 cm and the bag was sealed until the end of microwave heating. The tray with the sample then was placed in the center of the microwave chamber. The sample was heated at 100% power for 3 min. At the end of 3 min, the microwave door was opened and a hole was made in the bag so that a thermometer could be inserted into the sample. The temperature of the sample was 107 ± 2° C. The sample was cooled to room temperature (25°C). This was repeated until there was sufficient bran stabilized with microwave heat for the experiments. The samples were stored in an ultra freezer (-78 to -80°C) until the day of packaging (within 2 days).

Packaging and Storage of Rice Bran. Microwave heat stabilized and raw rice bran were divided in half, and from the first half, ten representative samples, each weighing 70-75g, were packed in polyethylene Ziplock® bags (Hefty One Zip multi purpose storage bag, Quart size, Mobile Chemical Co., NY), yielding a total of 20 bags. The bags were marked for storage time of 0, 4, 8, 12, and 16 weeks.

The other half were placed in an ice box, covered with dry ice, and delivered to the LSU Animal Science Department to be vacuum packed. The temperature of the room was 4-5° C. Ten representative samples, each weighing 70-75g, prepared from raw and microwave stabilized bran, were placed in non-permeable vacuum bags and vacuum packed. The vacuum machine used was TurboVAC (Model SB600, Howden Food Equipment, Netherlands). The bags were marked for storage time of 0, 4, 8, 12, or 16 weeks. The samples were brought back to the PBRC laboratory in the ice box . All bags were also marked for FFA, fatty acid composition, LOX activity, and

proximate analysis (at 0 and 16 weeks of storage). Half of the bags (20 bags total, 5 of each treatment) were stored in the refrigerator (3-4°C) and the other half (20 bags) at room temperature (25°C). Room temperature and the temperature in the refrigerator were monitored and recorded daily. Figure 15 shows the schematic of rice bran processing and storage. Approximately 2,450g rice bran was used for this experiment.

Fatty Acid Composition. The fat from the bran samples in the Ziplock® bags verses vacuum packed bags stored at room temperature verses refrigerated temperature was extracted with petroleum ether (Soxtec apparatus). The fatty acid composition was determined in duplicate by Gas Chromatography (GC) according to the American Oil Chemist Society method # Ce 1b-89 (AOCS, 1991) with modifications. After extracting the fat and dissolving it in 10 ml of petroleum ether, 100 µl was saved in a glass tube in the freezer (-25°C) until the day of analysis. To each sample 100 µl of internal standard (IS) was added. The solvent was evaporated on a N-EVAP (Model # 112, Organomation Associate Inc., MA) which was equipped with a water bath and was set between 40-45°C for faster evaporation of solvent under nitrogen gas. Then 1.5 ml of 0.5 N NaOH was added to each sample, blanketed with nitrogen, capped tightly, mixed, and heated with a vortex/heater(Model # 432-2000, Buchler Instruments, A Labconco Co., MO) at 100°C for 5 min. The samples were cooled and 2 ml of BF3/Methanol reagent (Boron Trifluride-methanol 14% solution, # B-1252, Sigma Chemical Co., MO) was added to each sample, blanketed with nitrogen, capped tightly, mixed and heated at 100°C for 30 min. The samples were cooled to 30-40°C and 1 ml of iso-octane was added to each sample. The samples were blanketed with

nitrogen, capped tightly, and vortexed (Vortex-Genie-2) for 30 seconds. Immediately, 5 ml of saturated NaCl (ACS #832-007, Curtis Matheson Scientific, Inc., TX) was added, blanketed with nitrogen, capped tightly, and agitated. The samples were cooled at room temperature until the iso-octane layer was separated from the aqueous layer. The iso-octane layer was transferred to another clean marked tube using a pasture glass disposable pipette. The samples were blanketed with nitrogen and capped. The methanol/water phase was extracted again with an additional 1 ml of iso-octane and the two extracts were combined and evaporated to 1ml. The extract was transferred to a GC vial (12x32 CLR vial, 11 mm, wide mouth, Allometrics, Inc., LA) using a pasture glass pipette, capped (11 mm, Alum, Seal w/TFE-SI, Allometrics, Inc., LA), and then run immediately.

Internal Standard (IS) used was C:23 (# N-23M, NuChek Prep Inc., MN) and was prepared by weighing 25 mg of C:23 into a 25 ml volumetric flask and brought to volume with iso-octane (2'2'4-Trimethylpetane, ultra resi, # 9335-02, J.T. Baker Inc., NJ). The calibration standard (#GLC-85, NuChek Prep Inc., MN) of fatty acids methylesters was prepared by emptying the content of ampoule received (100 mg) in 10 ml volumetric flask and bringing to volume with hexan. Further dilutions were made to get the concentration of 1 mg/ml.

The GC used was a Hewlett Packard # 5890 with autosampler also equipped with a Flame Ionization Detector (FID). The gases used were hydrogen as the fuel source for the detector, helium, and air. The column used was fused silica capillary column 30 m in length, 0.25 mm ID, and 0.20μm film thickness (Supelco # SP-2380,

Supelco, Inc., PA). The initial column temperature was programmed at 50°C, held for 1 min, then increased at 10°C/min to 150°C and held for 10 min, increased at 2°C/min to 175°C and held for 10 min, and increased at 5°C/min to 225°C and held for 7 min. The injector and detector port temperatures were maintained at 170°C and 270°C, respectively, and helium was used as a carrier gas. Fatty acids were identified by comparing their retention times with a standard mixture containing 30 fatty acid methyl esters (GLC-85, NuChek Prep Inc., MN)

In a GC vial 300 µl of GLC-85 standard solution,100 µl of IS and 600µl of iso-octane were added. The concentration of each fatty acid methylester was calculated (according to the data sheet provided by the company) and entered in the calibration table in Gas Chromotogragh (GC) computer. In addition, the amount of lipid extracted from each sample and the multiplier were entered in the computer. Fatty acid contents were quantified based on peak areas of known concentration of respective standards obtained under identical conditions. Recovery (%) of internal standard was also taken into consideration.

Proximate Analysis. Nutritional data such as protein, fat, ash, and carbohydrate in rice bran were determined in duplicate and on the wet basis at 0 weeks and the end of the storage period (16 weeks) in both vacuum and Ziplock® packed samples and two storage temperature (25°C vs. 4-5°C).

- **Protein.** Protein in rice bran samples was determined in duplicate using a nitrogen analyzer (Perkin Elmer, # PE 2410 series II). 100-125 mg of rice bran samples were used (AOAC method # 992.5). The instrument combusted the samples

to release nitrogen, which was then analyzed by thermal conductivity. Protein was calculated from the nitrogen content by using a conversion factor of 6.25 (AOAC, 1991). This calculation was done automatically by the instrument.

• **Fat.** Fat was determined in duplicate using AOAC Official Method 945.16A (AOAC, 1991). Approximately 2 g of samples were combined with a small amount of Ottawa sand standard (# S23-3, Fisher Scientific Co., PA) in a 26 mm cellulose thimble (# 1522-0018, Fisher Scientific Co., PA) and placed in the oven at 125°C for 1 hr. The fat from the sample was extracted with 35 ml petroleum ether (# 9268-03, J.T. Baker Inc., NJ) using a Soxtec System HT apparatus (Model # 1043 Extraction Unit, Perstorp Analytical Inc., MD).

Percent fat was determined by using the equation below:

% Fat = [final cup weight (g) - initial cup weight (g)[/ sample weight (g) x 100.

• **Moisture.** Moisture content was determined in duplicate using AOAC Official Method 985.14 (AOAC, 1991). Approximately 1 g of sample was placed on a fiber sample pad (CEM # 200150, CEM Corporation. NC) and microwaved at 90 % power for 5 min in a microwave solid analyzer (Lab Wave 9000, CEM Corporation, NC). Percent moisture was determined by weighing the sample until a constant weight was achieved during microwave heating. Calculation shown below was done automatically by the instrument:

% moisture = [(initial weight of sample (g) + sample-pad (g) before heating) - (weight of sample (g) + sample-pad (g) after heating)] / initial weight of sample (g) x 100.

• **Ash.** The method used was based on AOAC Official Methods 920.153, 900.02, and/or 923.03 (AOAC, 1991). Approximately 0.5-1 g of sample was placed into a fiber crucible (3 PN/303040 20 ml in size, CEM Corporation, NC) and was incinerated at 550°C for 2 hrs in a microwave muffle furnace (MAS 7000, CEM Corporation, NC). Ash was determined in duplicate by manually weighing the final product. Percent ash was calculated automatically by the instrument using the calculation below:

% Ash = [(initial weight of sample (g) + crucible before ashing) - (weight of sample (g) + crucible (g) after ashing)] / initial weight of sample (g) x 100.

• **Carbohydrate.** Percent carbohydrate was determined by difference using the formula below:

% Carbohydrate = 100 - (% protein + % fat + % moisture + % ash).

Statistical Analysis. A completely randomized design was used. To study the main effect for each factor, three factor factorial (2x2x2) and four factor factorial (2x2x2x4) arrangements (Table 21 in Appendices) were used for each variable. To compare the mean of the results (Table 22 and 23 in Appendices), the Student-Newman-Keuls (SNK) test was done (generally this test declares more significant differences than other tests such as Tukey's procedure). A statistical analysis of variance (ANOVA) was performed on all values using the Statistical Analysis System (SAS®) program version 6.12 (SAS, 1997). Differences were considered significant when means of compared sets differed at the $p < 0.05$ level of significance.

Results and Discussions. The results obtained in this study are as follows:

Fatty Acid Content. Distribution of fatty acids (Table 3) did not show significant ($p > 0.05$) difference between raw and microwave heat stabilized rice bran at 0 week, and during 16 weeks of storage except for the C18:1 content of raw ZRT and ZRef at 16 week storage. Oleic acid (C18:1), linoleic acid (C18:2), and palmitic acid (C 16:0) are dominant fatty acids (ca. 48%, 32%, and 14 % respectively) (Table 3) in raw and microwaved rice bran. The level of these fatty acids are dependent on the variety and the location of the plant growth (Saunders, 1990; Gupta, 1989). Distribution of fatty acids agreed with data reported by Saunders (1990). Table 4 shows fatty acid composition of rice bran oil and other common vegetable oils (Saunders, 1990).

Palmitic acid of raw and microwave heat stabilized bran increased from about 14% at week 0 to 17% at week 16, except the microwave VRT samples (Table 3). This could be due to the bound lipids which were released during the microwave heating and traveled into the oil during extraction (Krishnamurthy and Nagaraja ,1989). Bound lipid are normally rich in unsaturated fatty acids. Consequently the reletive weight percentage of oleic acid has come down compared to control samples (Krishnamurthy and Nagaraja, 1989). The main effect and interaction of the factors was not significant. Stearic acid and linolenic acid did not change significantly. Oleic acid content decreased after 16 weeks of storage in both raw and microwave heat stabilized samples; significant decreases were observed for raw rice bran stored in the Ziplock® bags, irrespective of storage temperature. There was a decrease in linoleic

Table 3. Fatty acid composition of microwave (M) heat stabilized and raw (R) rice bran at 0 and 16 weeks of storage[a].

			Fatty acid %				
			$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Raw(Control)	0 week		14.5 ± 0.5[c]	2.3 ± 0.2[a]	47.9 ± 0.2 [a]	32.4 ± 0.5[a]	1.1 ± 0.0 [a]
	16 weeks	ZRT	19.9 ± 0.1[a]	2.7 ± 0.0[a]	42.0 ± 0.2 [c]	32.4 ± 0.1[a]	1.2± 0.0 [a]
		ZRef	17.1 ± 3.0[b]	2.6 ± 0.5[a]	45.0 ± 4.0 [b]	30.5 ± 4.3[a]	1.1 ± 0.2 [a]
		VRT	17.0 ± 2.0[b]	2.7 ± 0.3[a]	45.5 ± 2.0 [ab]	33.4 ±0.7[a]	1.1 ± 0.2 [a]
		VRef	18.5 ± 0.2[ab]	2.5 ± 0.0[a]	45.9 ± 0.1[ab]	30.2 ± 0.1[a]	1.1 ± 0.0 [a]
Microwave	0 week	0 week	14.3± 0.1[c]	2.2 ± 0.1[a]	48.0 ± 0.1 [a]	32.6 ±0.2 [a]	1.1 ± 0.0 [a]
	16 weeks	ZRT	18.1 ± 0.5[ab]	2.5 ± 0.1[a]	46.7 ± 0.5 [ab]	29.9 ±0.3 [a]	1.1 ± 0.0 [a]
		ZRef	18.1 ± 0.4[ab]	2.5 ± 0.1[a]	46.0 ± 0.3 [ab]	30.3 ± 0.2[a]	1.2 ± 0.0[a]
		VRT	14.7 ± 0.3[c]	2.8 ± 0.0[a]	44.2 ± 0.2 [ab]	34.9 ± 0.1[a]	1.3 ± 0.0[a]
		VRef	18.3 ± 0.0[ab]	2.4 ± 0.0[a]	45.9 ± 0.0[ab]	30.4 ± 0.0 [a]	1.2 ± 0.0 [a]

[a]Means (average of 2 values) within a column with different letters are significantly different ($p < 0.05$).
Z = Ziplock® bag, V = vacuum pack, RT = room temperature, Ref = refrigerator

Table 4. Fatty acid composition of rice bran oil and other common vegetable oil.

	Fatty acid %				
Oil	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Rice bran	17	2	40	34	1
Palm	17	4	37	9	0
Peanut	14	3	39	36	0
Cottonseed	23	3	17	51	0
Corn	11	2	24	58	1
Soybean	10	4	23	51	1
Safflower	6	2	12	74	1

From Saunders (1990).

acid but these changes were not significant. Linoleic acid, the best substrate for LOX, decreased from an initial value of 32.6 % to 29.9% in microwave heat stabilized samples after 16 weeks of storage. Table 5 shows there was no significant ($p < 0.05$) change in linoleic acid at each storage interval. Hafez et al. (1985a) noticed there were no quantitative differences in fatty acid composition of raw and microwave heat stabilized soybeans after microwaving the samples for 15 min, but protein digestibility decreased. Yoshida et al. (1991) reported that microwave heat acts differently on fatty acid composition from tocopherols: the higher the degree of unsaturation, the greater the chemical changes in ethylesters. The ethyl linoleate showed the highest chemical changes. In this study, microwave heat stabilized samples showed more change in unsaturated (oleic and to a lesser extent, linoleic acid) than other fatty acids. Hafez et al. (1985b) noticed no significant ($p < 0.05$) changes in fatty acids (C16:0, C18:0, C18:1, and C18:2) at different radiation doses of γ irradiation of soybean. They reported that high radiation doses caused a decrease in linoleic acid. Increases in moisture content and radiation dose did not effect fatty acids except for a reduction in linoleic acid. γ irradiation of rice seeds with intact hull minimized the increase in the amount of unsaturated fatty acids in the FFA, which are prone to oxidation in the presence of oxygen radicals generated by γ irradiation.

Proximate Composition. Proximate composition of raw and microwave heat stabilized rice bran was obtained and analyzed (Table 6). There were no significant ($p > 0.05$) changes in protein and fat during storage. These data agree with the earlier findings (Wadsworth and Koltun, 1986; Yeo and Shibamoto, 1991).

Table 5. Effect of microwave heat stabilization on % linoleic acid of rice bran packed in Ziplock® bags (Z) and vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

		Storage Time (weeks)				
		0	4	8	12	16
		Linoleic Acid %				
Raw(Control)		32.4 ± 0.1[a]	32.4 ± 0.1[a]	32.4 ± 0.1 [a]	32.4 ± 0.1 [a]	32.4 ± 0.1[a]
	ZRT		34.4 ± 1.0[a]	33.1 ± 0.4[a]	31.0 ± 0.6[a]	32.4 ± 0.1[a]
	ZRef		32.1 ± 1.9 [a]	32.8± 0.3[a]	31.5 ± 0.1[a]	30.5 ± 4.2[a]
	VRT		33.9 ±0.0[a]	32.3±0.5[a]	31.4 ± 0.2[a]	33.4 ± 0.6[a]
	VRef		33.3 ± 0.2 [a]	33.0±0.7[a]	31.7± 0.1[a]	30.2 ±0.1[a]
Microwave		32.6± 0.1[a]	32.6± 0.1[a]	32.6 ± 0.1[a]	32.6 ± 0.1[a]	32.6 ± 0.1[a]
	ZRT		34.0 ± 0.1[a]	33.4 ±0.5[a]	32.2 ± 1.1[a]	29.9 ± 0.3[a]
	ZRef		33.5 ± 0.0[a]	33.4 ± 0.7[a]	31.0 ± 1.2[a]	30.3 ± 0.2[a]
	VRT		33.7 ±0.2[a]	33.1 ± 0.5[a]	31.6 ± 0.1[a]	34.9 ± 0.1[a]
	VRef		33.7 ± 0.1[a]	34.1± 0.0[a]	31.8 ± 0.1[a]	30.4 ±0.0[a]

[a]Means (average of 2 values) between columns with different letters are significantly different ($p < 0.05$).

Table 6. Proximate composition of rice bran packed in a Ziplock® bags (Z) and vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

			Protein (%)	Fat (%)	Moisture (%)	Ash (%)	Carbohydrate (%)
Raw(Control)	0 week		17.1 ± 0.6[a]	16.4 ± 0.0[a]	7.5 ± 0.1[b]	7.4 ± 0.1[bc]	51.7 ± 0.7[a]
	16 weeks	ZRT	17.4 ± 0.1[a]	16.9 ± 0.0[a]	7.0 ± 0.0[bc]	8.3 ± 0.2[a]	50.4 ± 0.0[b]
		ZRef	17.0 ± 0.2[a]	17.6 ± 1.1[a]	9.3 ± 0.1[a]	7.9 ± 0.1[ab]	48.2 ± 0.6[c]
		VRT	17.5 ± 0.2[a]	16.9 ± 0.2[a]	6.5 ± 0.2[c]	8.4 ± 0.2[a]	50.8 ± 0.4[b]
		VRef	17.0 ± 0.1[a]	16.9 ± 0.2[a]	8.5 ± 0.2[a]	7.8 ± 0.3[bc]	49.8 ± 0.7[bc]
Microwave	0 week		17.5 ± 0.4[a]	17.5 ± 0.4[a]	8.4 ± 0.4[a]	7.6 ± 0.1[bc]	48.9 ± 0.3[bc]
	16 weeks	ZRT	17.7 ± 0.4[a]	17.9 ± 0.3[a]	6.4 ± 0.2[c]	8.4 ± 0.1[a]	49.7 ± 0.5[bc]
		ZRef	17.9 ± 0.3[a]	17.3 ± 0.0[a]	6.3 ± 0.1[c]	8.2 ± 0.1[a]	50.3 ± 0.3[b]
		VRT	18.0 ± 0.1[a]	17.0 ± 0.1[a]	6.3 ± 0.6[c]	8.4 ± 0.1[a]	50.4 ± 0.6[bc]
		VRef	17.5 ± 0.3[a]	17.1 ± 0.1[a]	7.6 ± 0.7[b]	8.3 ± 0.0[a]	49.5 ± 0.9[bc]

[a]Means (average of 2 values) within a column with different letters are significantly different ($p \leq 0.05$).

Moisture content was significantly ($p < 0.05$) higher in microwave heat stabilized samples than raw samples, 8.4% and 7.5%, respectively, at 0 week. Raw samples with moisture content of 7.5% were adjusted to 21% before microwave heating. After microwave heating the moisture content was measured and it was 8.4%. The color of the raw bran was light tan and after microwave heating the color was darker than raw with a toasted aroma. These changes could be because of a slight browning reaction. According to Yeo and Shibamoto (1991), browning intensities of an L-cysteine/D-glucose model system with microwave irradiation for 2.5 min at 22% moisture content showed, a significant browning compared to 14% moisture content. They reported that the reduction in the moisture content (before and after microwaving) and production of darker color is because at the initial stage of irradiation. The source of energy is solely or predominantly due to microwave irradiation, as there are more polarized dipoles (such as water) to undergo rotation and absorb microwave energy. However, as the irradiation proceeds, the source of energy is probably due to microwave and thermal effects. In addition, as the irradiation proceeds, water is removed from the system. This dehydration process favors the formation of brown color and loss of moisture in our samples.

In raw samples the moisture content increased significantly ($p < 0.05$) in the samples packed in Ziplock® bags and vacuum packed and stored in the refrigerator, while the samples stored at room temperature had a slight decrease in the amount of moisture. However, in the microwave stabilized samples the moisture content decreased significantly ($p < 0.05$) for all samples, which could also be a contributing

factor for increase in LOX activity of samples stored at room temperature. Ash and carbohydrate showed significant fluctuations. The amount of ash increased from an initial concentration of 7.6% to 8.2-8.4% in microwave heat stabilized samples. Microwave heat had a slight effects on mineral content of microwave baked potatoes (Klein and Mondy, 1981). Carbohydrate showed significant ($p < 0.05$) decrease from an initial value of 51.8 to 48.2% in raw samples packed in Ziplock® bags and stored in the refrigerator. However, the microwave heat stabilized sample started (0 week) at significantly lower carbohydrate content than the raw samples and did not change significantly during 16 weeks of storage (Table 6). The results from this study agreed with previous findings (Tsen et al., 1977; Malekian, 1992). Microwave heat had little effect on the proximate composition of rice bran packed in the Ziplock® bags and vacuum packed and stored in the refrigerator for 8 weeks (Malekian, 1992), as well as the breads made with soy and wheat flour (Tsen et al., 1977).

Conclusions. Rice bran is the outer brown layer of brown rice which is removed through the milling process. Right after milling, if the bran is subjected to high temperatures for a short time (microwave heating), stabilized rice bran is produced which is very nutritive. Fatty acid and proximate composition did not change drastically in microwave heat stabilized samples compared to raw samples kept in identical storage conditions. The optimum conditions for these variables would be microwave heating, packing in Ziplock® bags, and storing in the refrigerator (4-5°C).

CHAPTER 6
EFFECT OF PACKAGING AND STORAGE ON SHELF-LIFE OF MICROWAVE HEAT STABILIZED RICE BRAN

Introduction. Rice bran, the term applied to the coating removed from brown rice during milling, is composed of seed coat, the major part of the germ, and most of the outer layer of the kernel together with some broken kernel. Rice bran, which constitutes about 7-8.5% of the whole grain, is highly nutritious, with a protein content of 12-16%, fat content of 16-22%, and providing a good source of vitamins (B complex, and E) and minerals. Rice bran is a good source of oil (Saunders, 1990). A major difficulty with rice bran as a raw material is the protection of high quality edible oil and its deterioration in storage after milling, principally as a result of hydrolysis (lipase activity) and oxidation (lipoxygenase activity and autoxidation). Perhaps the most reliable method for testing rancidity development is tedious periodic evaluation of stored samples under actual commercial packaging conditions. There are so many factors contributing to rancidity. Knowing these factors and how to eliminate them could extend the shelf-life of rice bran. Because oxidation factors can be introduced in so many ways, elimination of rancidity may be impossible. The packaging (seams and seals, vacuum, coating, etc.), the product itself (presence of oxidizing accelerator, metallic ions, certain enzymes, random changes to fats and oil, elimination of antioxidant, microbial factors, etc.), and environmental factors (temperature, pH, and related processing) could be controlled. Lipase activity during storage is the factor for the overall extent of degradation (production of FFA) of lipids in bran during storage. The LOX will oxidize polyunsaturated fatty acids but is dependent on the earlier

release of polyunsaturated fatty acids from triglycerides by lipases. This decomposition results in not only free acids but also a bad taste that reduces the marketing potential of rice bran. Values for FFA acids present is widely used as a quality indicator for fats and oils. Heat treatment is mostly used to denature the enzymes and stabilize a product. Various methods of brown rice and rice bran storage have been used to determine a temperature, atmosphere, and kind of packaging that prolong shelf-life. Storage at a cool temperature has been shown to slow down deterioration of lipid compounds in brown rice (Loeb et al., 1949; Sharp and Timme, 1986). Packaging can be used to influence the storage stability of rice bran. Packing milled rice in a polyethylene and nylon combination bag was found to be most suitable for long-term storage of milled rice under vacuum and with carbon dioxide flushing (Sharp and Timme, 1986).

This study was undertaken to examine the effect of: two types of packing (Ziplock® bags and vacuum packing), two storage temperatures (room temperature, ca. at 25°C and refrigeration ca. 5°C), and storage time (16 weeks) on the shelf-life of rice bran. The specific objectives were:

1. To study the effect of microwave heat on the shelf-life (16 weeks) of rice bran
2. To study the effect of packaging methods (vacuum vs. Ziplock® bags) on the shelf-life (16 weeks) of rice bran
3. To study the effect of temperature (25°C vs. 4-5°C) on the shelf-life (16 weeks) of rice bran

Materials and Methods. Material and methods are as follows:

Rice Bran Collection. Rice variety 'Lemont' (long grain), cultivated at the Louisiana Rice Experiment Station, Crowley, Louisiana was used for this experiment. The rice samples were dehusked and milled (friction type) by a Satake milling system (Satake USA Houston, TX) at the Biological and Agricultural Engineering Department at Louisiana State University. Rice bran was collected in a barrel lined with a black plastic bag. Dry ice was added continuously to rice bran in the barrel during milling to prevent the hydrolysis of fatty acids by lipase activity. The bag was tied tightly and was delivered (within 15 min) to the Pennington Biomedical Research Center (PBRC) laboratory. The bags were placed in the Ultra freezer (-78 to -80 C°) until the day of sample preparation (within 10 days). On the day of sample preparation, the samples were sieved with a 20 mesh siever in order to remove broken pieces of rice and husks,. A thermometer was placed in rice bran samples to monitor the temperature (0-2°C) during the sieving process.

Microwave Heat Stabilization. One hundred-fifty g per batch of raw rice bran were stabilized with a Sharp Carousel microwave oven (Model R3A96, Sharp Electronic Corporation, NJ) 850 Watts power at 2450 MHz. The microwave chamber was heated before the stabilizing process by running the microwave at high for 3 min. The moisture content of raw rice bran samples was adjusted on wet bases from initial 7.0 % to 21 % by using the calculation below:

$$150g \times 0.07 = 10.5ml$$

$$10.5\ ml + X = 0.21(150g + X)$$

$10.5 \text{ ml} + X = 31.5\text{g} + 0.21\ X$

$X = 26.58 \text{ ml}$

Therefore, 26.58 ml of deionized (DI) water were added to 150 g of rice bran sample to obtain 21% moisture content; the percentage of moisture content has been optimized in previous studies (Tao, 1989; Malekian, 1992). The sample was mixed thoroughly with a stainless steel spatula to make sure that the water was evenly distributed. Each sample was placed in a Ziplock® one gallon storage bag (Hefty One Zip multi purpose storage bag Gallon size, Mobile Chemical Co., NY) and spread out evenly to a thickness of 0.5 cm and the bag was sealed until the end of microwave heating. The sample was heated at 100% power for 3 min. At the end of 3 min, the microwave door was opened and a hole was made in the bag so that a thermometer could be inserted into the sample. The temperature of the sample was 107 ± 2° C. The sample was cooled to room temperature (25°C). This was repeated until there was sufficient bran stabilized with microwave heat for the experiments. The samples were stored in an ultra freezer (-78 to -80°C) until the day of packaging (within 2 days).

Packaging and Storage of Rice Bran. Microwave heat stabilized and raw rice bran were divided in half, and from the first half, ten representative samples, each weighing 70-75g, were packed in polyethylene Ziplock® bags (Hefty One Zip multi purpose storage bag, Quart size, Mobile Chemical Co., NY), yielding a total of 20 bags. The bags were marked for storage time of 0, 4, 8, 12, and 16 weeks.

The other half was placed in an ice box, covered with dry ice, and delivered to the LSU Animal Science Department to be vacuum packed. The temperature of the room was 4-5° C. Ten representative samples, each weighing 70-75g, prepared from raw and microwave stabilized bran, were placed in non-permeable vacuum bags and vacuum packed. The vacuum machine used was TurboVAC (Model SB600, Howden Food Equipment, Netherlands). The bags were marked for storage time of 0, 4, 8, 12, or 16 weeks. The samples were brought back to the PBRC laboratory in the ice box . All bags were also marked for FFA, fatty acid composition, LOX activity, and proximate analysis (at 0 and 16 weeks of storage). Half of the bags (20 bags total, 5 of each treatment) were stored in the refrigerator (4-5°C) and the other half (20 bags) at 25°C. Room temperature and the temperature in the refrigerator were monitored and recorded daily. Figure 15 shows the schematic of rice bran processing and storage. Approximately 2, 450g rice bran was used for this experiment.

Free Fatty Acid Determination. Free fatty acids were determined in duplicate using the method of Hoffpauir et al. (1947) modified to use m-cresol purple instead of phenolphthalein as an indicator.

Alcoholic sodium hydroxide was prepared by adding one pellet of NaOH (#3722-01, J.T. Baker Inc., NJ) to 500 ml of absolute ethanol in a flask. The top was covered tightly and the mixture was mixed on a stirrer/hot plate for 30-60 min or until the sodium hydroxide pellet (0.084-0.1035) was completely dissolved. A stock solution of m-cresol purple was prepared by dissolving 0.1 g of m-cresol purple (#C-1393, Sigma Chemical Co, MO) in 24 ml of 0.01 N NaOH solution, and was diluted to 250

ml with DI water. The working solution of m-cresol was prepared by mixing 1 ml of stock solution with 100 ml of absolute alcohol. Approximately 1 ml of alcoholic sodium hydroxide was added by titration to the m-cresol purple working solution to reach a grayish purple endpoint. To standardize alcoholic NaOH, 1 ml of 0.1 N hydrochloric acid was added to 25 ml of m-cresol purple working solution and 10 ml of petroleum ether in a 250 ml Erlenmeyer flask. This solution was titrated with the alcoholic sodium hydroxide to a purplish gray endpoint. The concentration of alcoholic sodium hydroxide was calculated by dividing 0.1 by the volume of the alcoholic sodium hydroxide used for the titration. The blank was determined by titrating 25 ml of m-cresol purple working solution plus 10 ml of petroleum ether with the alcoholic sodium hydroxide.

The fat was extracted in duplicate from samples with a Soxtec System HT apparatus (Model 1043 Extraction Unit, Perstorp Analytical Inc., MD). The extracted fat was dissolved in 10 ml of petroleum ether in a flask, then 25 ml m-cresol purple working solution was added to this flask. The content of the flask was titrated with alcoholic sodium hydroxide .

Percent FFA was determined using the calculation below:

% FFA = (ml titrated - ml blank) x 28.2 x concen. alc. NaOH / lipid weight (g)

ml titrated = the ml of alcoholic NaOH which was used to change the color of the above solution (sample solution) to grayish purple at the endpoint, ml blank = the ml of alcoholic NaOH which was used to change the color of blank solution to grayish purple at the endpoint, 28.2 = multiplication factor obtained by multiplying the

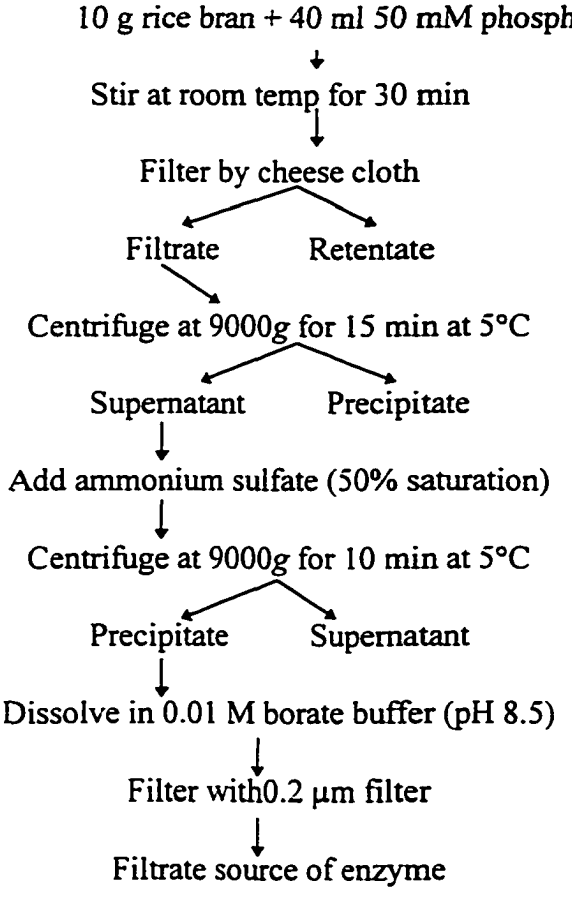
molecular weight of oleic acid (282.0) times the percentage (100) and divided by 1000 (ml in 1 L of solution), and concen.alc. NaOH = 0.1/ml of NaOH used to standardize.

Lipoxygenase Activity. LOX activity was determined in duplicate using the methods as described by Shastry and Raghavendra Rao. (1975), Aurand et al. (1987), and Dixon and Webb (1961) with modifications.

Standard Enzyme Preparation. Soybean lipoxygenase (# L 7395 lot # 118 F03422) was purchased from Sigma Chemical Co., MO. This standard enzyme contained 110,600 units per mg solid. An enzyme solution was made by adding 11.6 ml of 0.01 M borate buffer, pH 8.5 (Corning pH meter # 340, Corning Inc., NY) to 1mg dried standard enzyme to obtain 10,000 units of enzyme per ml of buffer. The enzyme standard solution was used for each analysis as a control.

Enzyme Extraction from Rice Bran Samples. In a beaker, 10 g of rice bran sample was mixed with 40 ml of 50 mM phosphate buffer (dibasic sodium phosphate, anhydrous, # 3827-01, J.T. Baker Inc., NJ) pH 7.0, for 30 min at room temperature. The sample was filtered into another beaker using two layers of cheese cloth. The filtrate was collected in a 50 ml (114 X 29 mm) graduated plastic conical tube (# 62.547.004, Sarstedt inc., NC) and was centrifuged (Model # J2- HC, Beckman Instrument Co., TX) at 9000*g* for 15 min at 5°C. The supernatant was collected in another 50 ml graduated plastic conical tube. The volume of the supernatant was recorded. Solid ammonium sulfate (A-2939, Sigma Chemical Co., MO) was added to each sample to obtain 50% saturation (Copper, 1942). The sample was mixed gently and centrifuged at 9000*g* for 10 min at 5°C. The volume was recorded. The

supernatant was discarded and the precipitate was dissolved in 0.01 M borate buffer (sodium borate S-9640, Sigma Chemical Co., MO) pH 8.5, and the volume was adjusted until the previously recorded volume was obtained for each sample. This solution was filtered with a UNIFLO 10 cc disposable syringe (# 309604, Becton Dickinson & Co., NJ) and filters (0.2 μm pore size, 25 mm in diameter non-sterile units, Schleicher & Schuell, NH), and the filtrate was used as the source of enzyme. For assay, the enzyme extract was diluted 1 to 1 with 0.01 mM borate buffer pH 8.5. The flow diagram of the LOX extraction follows:



Substrate Preparation. To a 100 ml volumetric flask, 100 µl of linoleic acid (# U-50-A, with purity greater than 99%+, NuChek Prep, Inc., MN) and 60 ml of absolute alcohol (200 proof, McCormick Distilling Co. Inc., MO) was added. The mixture was mixed gently into an emulsion and then, with slow stirring, DI water was added to bring the volume to 100 ml. This was used as a stock solution. For assay,1 ml of stock solution was diluted with 6 ml of 0.01 M borate buffer, pH 8.5 (flow diagram below). The concentration of linoleic acid with 1 to 6 dilution was 0.4571 mM per ml. The flow diagram of substrate preparation follows:

100 µl of linoleic acid
↓
Dissolve in 60 ml absolute alcohol (in 100 ml volumetric flask)
↓
Volume to 100 ml with DI water
↓
Stock solution of substrate
↓
1 ml of stock solution + 6 ml of 0.01 M borate buffer (pH 8.5)

Procedure for Determination of Lipoxygenase Activity. Enzyme activity was measured in duplicate with a thermostated spectrophotometer (Model # Du 640, Beckman Instrument Inc., TX). The time was set for 5 min, the temperature at 25°C (ambient temperature), and the absorbency (dA) at 234nm. Into two Quartz cuvettes, in 3 ml volume (1 cm light path), labeled "blank" and "sample ID", the following was pipetted:

	blank	sample
substrate solution	3.0ml	2.9ml
enzyme solution	0.0ml	0.1ml

The cuvette with 2.9 ml of substrate solution was placed in the cell of the spectrophotometer. The enzyme solution was added as rapidly as possible, mixed well, and the increase in dA_{234} versus the blank was recorded. One unit of LOX activity is defined as the change in absorbency of 0.001/min in 3 ml volume (1 cm light path) when linoleic acid is used as substrate (Shastry and Raghavendra Rao, 1975). The rate of increase is usually highest between 0.1-3 min, after which it decreased.

Fatty Acid Composition. The fat from the bran samples in the Ziplock® bags verses vacuum packed bags stored at room temperature verses refrigerated temperature was extracted with petroleum ether (Soxtec apparatus). The fatty acid composition was determined in duplicate by Gas Chromatography (GC) according to the American Oil Chemist Society method # Ce 1b-89 (AOCS, 1991) with modifications. After extracting the fat and dissolving it in 10 ml of petroleum ether, 100 µl was saved in a glass tube in the freezer (-25°C) until the day of analysis. To each sample 100 µl of internal standard (IS) was added. The solvent was evaporated on a N-EVAP (Model # 112, Organomation Associate Inc., MA) which was equipped with a water bath and was set between 40-45°C for faster evaporation of solvent under nitrogen gas. Then 1.5 ml of 0.5 N NaOH was added to each sample, blanketed with nitrogen, capped tightly, mixed, and heated with vortex/heater (Model # 432-2000, Buchler Instruments, A Labconco Co., MO) at 100°C for 5 min. The samples were cooled and 2 ml of BF3/Methanol reagent (Boron Trifluride-methanol 14% solution, # B-1252, Sigma Chemical Co., MO) was added to each sample, blanketed with nitrogen, capped

tightly, mixed and heated at 100°C for 30 min. The samples were cooled to 30-40°C and 1 ml of iso-octane was added to each sample. The samples were blanketed with nitrogen, capped tightly, and vortexed (Vortex-Genie-2) for 30 seconds. Immediately, 5 ml of saturated NaCl (ACS #832-007, Curtis Matheson Scientific, Inc., TX) was added, blanketed with nitrogen, capped tightly, and agitated. The samples were cooled at room temperature until the iso-octane layer was separated from the aqueous layer. The iso-octane layer was transferred to another clean marked tube using a pasture glass disposable pipette. The samples were blanketed with nitrogen and capped. The methanol/water phase was extracted again with an additional 1 ml of iso-octane and the two extracts were combined and evaporated to 1ml. The extract was transferred to a GC vial (12x32 CLR vial, 11 mm, wide mouth, Allometrics, Inc., LA) using a pasture glass pipette, capped (11 mm, Alum, Seal w/TFE-SI, Allometrics, Inc., LA), and then run immediately.

Internal Standard (IS) used was C:23 (# N-23M, NuChek Prep Inc., MN) and was prepared by weighing 25 mg of C:23 into a 25 ml volumetric flask and brought to volume with iso-octane (2'2'4-Trimethylpetane, ultra resi, # 9335-02, J.T. Baker Inc., NJ). The calibration standard (#GLC-85, NuChek Prep Inc., MN) of fatty acids methylesters was prepared by emptying the content of ampoule received (100 mg) in 10 ml volumetric flask and bringing to volume with hexan. Further dilution were made to get the concentration of 1 mg/ml.

The GC used was a Hewlett Packard # 5890 with autosampler also equipped with a Flame Ionization Detector (FID). The gases used were hydrogen as the fuel

source for the detector, helium, and air. The column used was fused silica capillary column 30 m in length, 0.25 mm ID, and 0.20μm film thickness (Supelco # SP-2380, Supelco, Inc., PA). The initial column temperature was programmed at 50°C, held for 1 min, then increased at 10°C/min to 150°C and held for 10 min, increased at 2°C/min to 175°C and held for 10 min, and increased at 5°C/min to 225°C and held for 7 min. The injector and detector port temperatures were maintained at 170°C and 270°C, respectively, and helium was used as a carrier gas. Fatty acids were identified by comparing their retention times with a standard mixture containing 30 fatty acid methyl esters (GLC-85, NuChek Prep Inc., MN)

In a GC vial 300 μl of GLC-85 standard solution,100 μl of IS and 600μl of iso-octane were added. The concentration of each fatty acid methylester was calculated (according to the data sheet provided by the company) and entered in the calibration table in Gas Chromotogragh (GC) computer. In addition, the amount of lipid extracted from each sample and the multiplier were entered in the computer. Fatty acid contents were quantified based on peak areas of known concentration of respective standards obtained under identical conditions. Recovery (%) of internal standard was also taken into consideration. Nutritional data such as protein, fat, ash, and carbohydrate were determined at 0 weeks and the end of the storage period (16 weeks) in both vacuum and Ziplock® packed samples.

Proximate Analysis. Nutritional data such as protein, fat, ash, and carbohydrate in rice bran were determined in duplicate on wet basis at 0 weeks and the end of the storage period (16 weeks) in both vacuum and Ziplock® packed samples.

•**Protein.** Protein in rice bran samples was determined in duplicate using a nitrogen analyzer (Perkin Elmer, # PE 2410 series II). 100-125 mg of rice bran samples were used (AOAC Official Method 992.5). The instrument combusted the samples to release nitrogen, which was then analyzed by thermal conductivity. Protein was calculated from the nitrogen content by using a conversion factor of 6.25 (AOAC, 1991). This calculation was done automatically by the instrument.

•**Fat.** Fat was determined in duplicate using AOAC Official Method 945.16A (AOAC, 1991). Approximately 2 g of samples were combined with a small amount of Ottawa sand standard (# S23-3, Fisher Scientific Co., PA) in a 26 mm cellulose thimble (# 1522-0018, Fisher Scientific Co., PA) and placed in the oven at 125°C for 1 hr. The fat from the sample was extracted with 35 ml petroleum ether (# 9268-03, J.T. Baker Inc., NJ) using a Soxtec System HT apparatus (Model # 1043 Extraction Unit, Perstorp Analytical Inc., MD).

Percent fat was determined by using the equation below:

% Fat = [final cup weight (g) - initial cup weight (g)[/ sample weight (g) x 100.

•**Moisture.** Moisture content was determined in duplicate using AOAC Official Method 985.14 (AOAC, 1991). Approximately 1 g of sample was placed on a fiber sample pad (CEM # 200150, CEM Corporation. NC) and microwaved at 90 % power for 5 min in a microwave solid analyzer (Lab Wave 9000, CEM Corporation, NC). Percent moisture was determined by weighing the sample until a constant weight was achieved during microwave heating.

Calculation shown below was done automatically by the instrument:

% moisture = [(initial weight of sample (g) + sample-pad (g) before heating) - (weight of sample (g) + sample-pad (g) after heating)] / initial weight of sample (g) x 100.

•**Ash.** The method used was based on AOAC Official Method 920.153, 900.02, and/or 923.03 (AOAC, 1991). Approximately 0.5-1 g of sample was placed into a fiber crucible (3 PN/303040 20 ml in size, CEM Corporation, NC) and was incinerated at 550°C for 2 hrs in a microwave muffle furnace (MAS 7000, CEM Corporation, NC). Ash was determined in duplicate by manually weighing the final product. Percent ash was calculated automatically by the instrument using the calculation below:

% Ash = [(initial weight of sample (g) + crucible before ashing) - (weight of sample (g) + crucible (g) after ashing)] / initial weight of sample (g) x 100.

•**Carbohydrate.** Percent carbohydrate was determined by difference using the formula below:

% Carbohydrate = 100 - (% protein + % fat + % moisture + % ash).

Statistical Analysis. A completely randomized design was used. To study the main effect for each factor, three factor factorial (2x2x2) and four factor factorial (2x2x2x4) arrangements (Table 9, 11, and 21 in Appendices) were used for each variable. To compare the mean of the results (Table 10, 12, 22, and 23 in Appendices), the Student-Newman-Keuls (SNK) test was done (generally this test declares more significant differences than other tests such as Turkey's procedure). A statistical analysis of variance (ANOVA) was performed on all values using the

Statistical Analysis System (SAS®) program version 6.12 (SAS, 1997). Differences were considered significant when means of compared sets differed at $p< 0.05$.

Results and Discussion. Statistical analysis of variance (ANOVA) was performed on all values. The *p*-values and corresponding type III sum of squares (Table 9 in Appendices) were used to describe the main effect and interaction among factors in the stored rice bran. The results showed that all the main effects were significant ($p <0.05$) for FFA, but for LOX, treatment of samples, storage temperature, and storage period were significant (main effect) and packaging methods were not significant. The interaction of the factors showed a different pattern. All the interactions of factors were significant ($p < 0.05$) for FFA, except for the interaction of treatment, types of packing, storage temperature, and length of storage. However, LOX activity showed a different pattern. All the main effects were significant ($p < 0.05$) but factor B (storage temperature), and all the interactions were not significant ($p > 0.05$) except for storage time (week) and factor C (packaging).

Extended storage resulted in the fluctuation of LOX activity. As a main effect, storage temperature showed there was a decrease in LOX activity in raw samples packed in Ziplock® bags and stored at room temperature (RZRT) at week 8, and a significant ($p < 0.05$) increase at week 12, and a significant ($p < 0.05$) decrease at week 16 (Figure 11). Vacuum packed samples did not show significant difference in LOX activity up to week 12, but a significant ($p <0.05$) decrease in LOX activity occurred after 12 weeks to 16 weeks of storage (Figure 11). However, FFA levels steadily increased throughout the storage period (Figure 12).

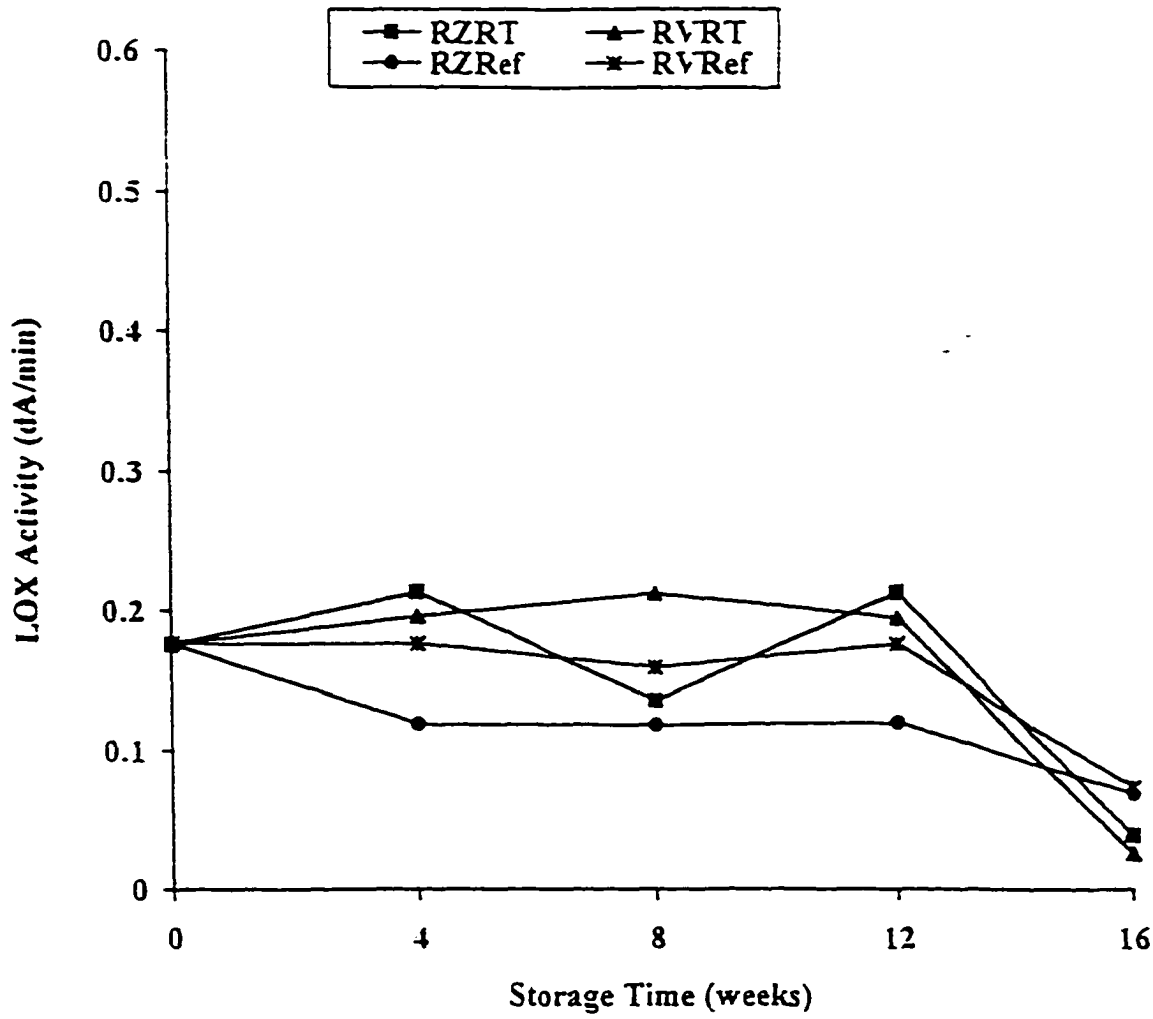


Figure 11. Lipoxygenase activity in raw (control) rice bran during 16 weeks of storage period packed in Ziplock® bags and vacuum packed, stored at 25°C and 4-5°C. Individual values, means and standard deviations presented in Table 17 in the Appendices. R= raw, Z= Ziplock® bags, V= vacuum packed, RT= room temperature, Ref= refrigerator

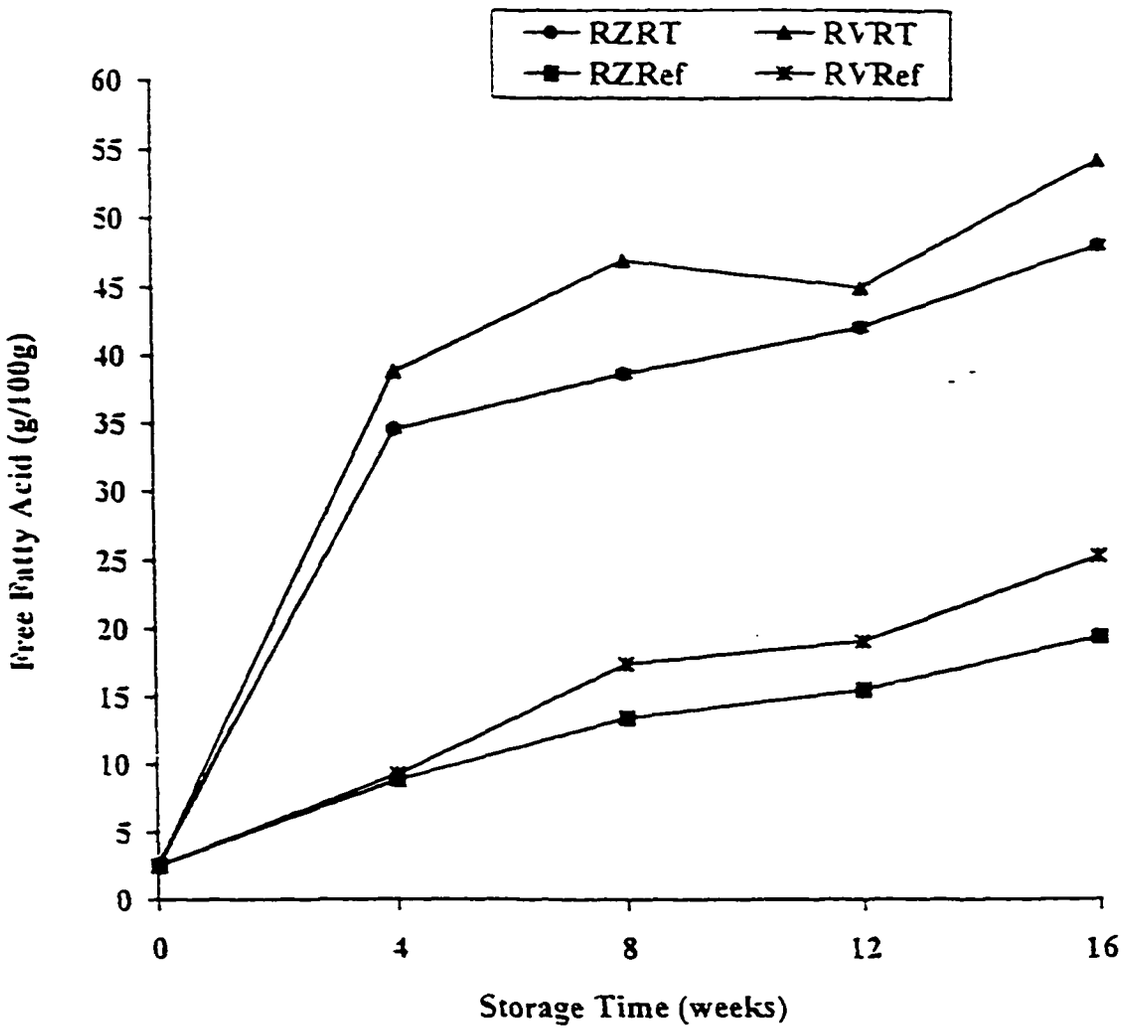


Figure 12.- Free fatty acid changes in raw rice bran during 16 weeks of storage. Individual values, means and standard deviations presented in Table 18 in the Appendices. R= raw, Z= Ziplock® bags, V= vacuum packed, RT= room temperature, Ref= refrigerator

FFA levels increased with increased in storage temperature and time. As the main effect, packaging method showed a difference in FFA levels between Ziplock® bags and vacuum packed samples. Without Exception, FFA level for samples in Ziplock® bags was lower than for vacuum packed samples (Figure 12). LOX activity did not show a difference between Ziplock® bags and vacuum packed samples. The interaction of storage time and storage temperature showed (Figure 11) that LOX activity for each storage temperature fluctuated (samples packed in Ziplock® bags and stored at room temperature) in different patterns as storage time progressed. FFA level showed (Figure 12) a steady increase except samples stored in the refrigerator had a lower percentage of free fatty acids than those stored at room temperature.

Interaction between storage time and packaging showed (Figure 11) that LOX activity fluctuated between Ziplock® bag and vacuum packed samples. As mentioned previously (storage and temperature), LOX activity for samples packed in Ziplock® bags increased at week 4, followed by a decrease at week 8, an increase up to 12 weeks, and a decrease up to 16 weeks of storage, while the vacuum packed samples remained unchanged until week 12 and decreased thereafter. However, FFA increased steadily throughout the storage period, and vacuum packed samples showed (Figure 12) more increase in FFA level than samples packed in Ziplock® bags regardless of the storage temperature.

Interaction between storage temperature and packaging had a significant effect on LOX activity and FFA levels. Raw bran samples packed in Ziplock® bags had more fluctuation in LOX activity as temperature increased than vacuum packed

samples. FFA levels increased steadily in the two different packaging methods as the storage temperature increased. Samples in Ziplock® bags had less increase in FFA levels than vacuum packed samples.

Data collected in this study show (Figure 12) that FFA levels in raw rice increased steadily in two different storage temperatures and two different packaging as storage period progressed. Samples packed in Ziplock® bags had less increase. FFA levels increased more slowly in samples stored in the refrigerator. LOX activity did not increase drastically except for samples packed in Ziplock® bags with a fluctuation in activity. LOX activity decreased significantly ($p < 0.05$) after 12 weeks of storage.

Microwave heat stabilized samples showed different patterns in main effects and interactions. In microwave heat stabilized samples, increases in FFA levels and LOX activity from increased temperature have been reported previously (Sharp and Timme, 1986; Piggott et al., 1991; Champagne et al., 1992). Figure 13 shows extended storage resulted in fluctuating LOX activity of microwave heated bran (not as drastic as in raw samples). FFA increased very slowly, and for samples stored in the refrigerator there was no significant change throughout the storage period (Figure 14). Sharp and Timme (1986) reported that LOX activity in brown rice had a peak at 2 and 7 months. In this study LOX activity in microwave heat stabilized samples packed in Ziplock® bags and stored at room temperature also showed sharp peaks at week 4 and at week 16. As storage progresses LOX activity changed from an initial value of 0.18 dA/min to 0.38dA/min at week 4, and decreased to 0.22 dA/min at week 8, and a drastic increase to 0.54 dA/min at week16 (Figure 13).

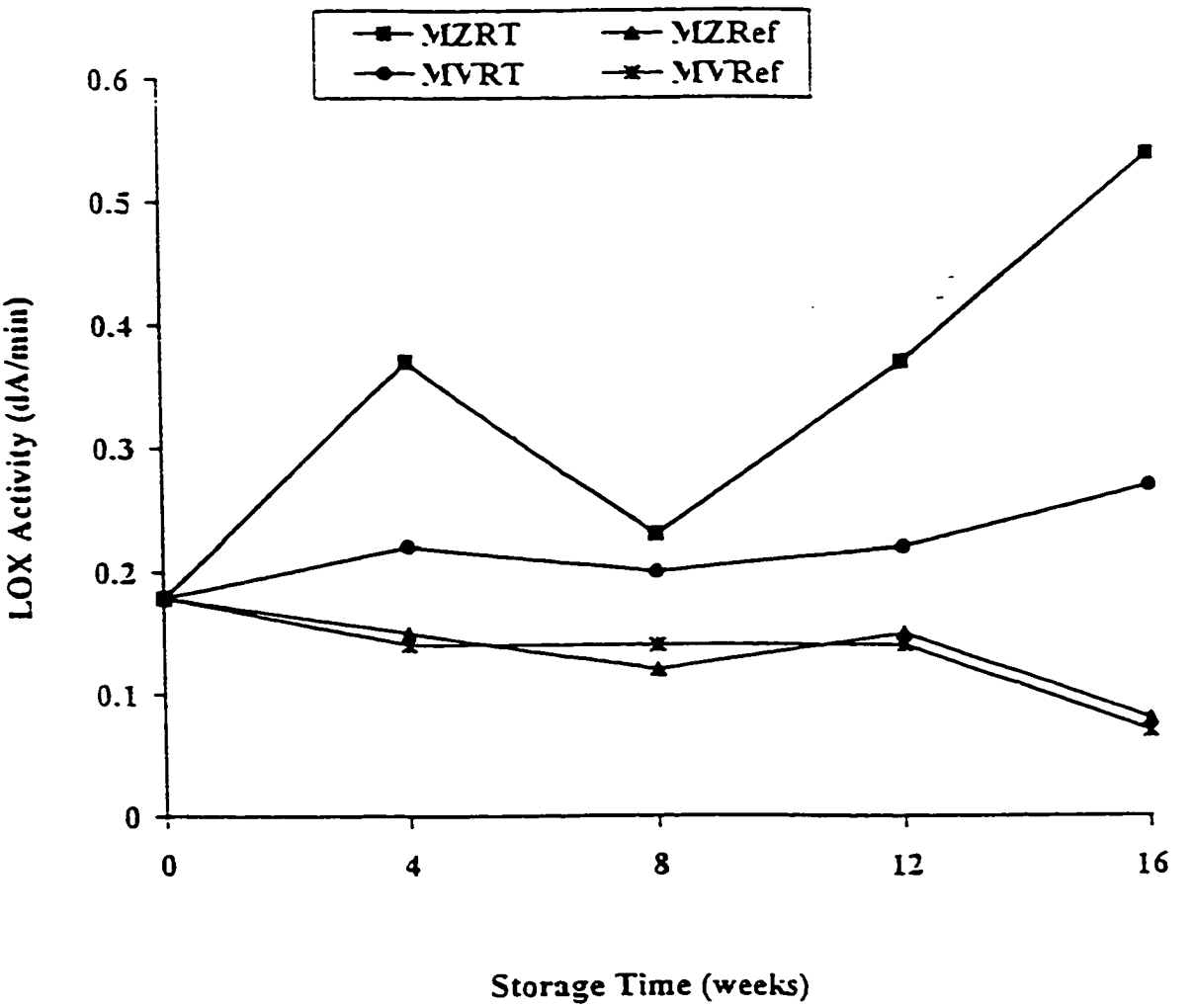


Figure 13: Lipoxygenase activity in microwave heat stabilized rice bran packed in Ziplock® bags and vacuum packed, stored at room temperature and refrigerator. Individual values, means and standard deviations presented in Table 19 in the Appendices. M= microwave heat stabilized, Z= Ziplock® bags, V= vacuum packed, RT= room temperature, Ref= refrigerator

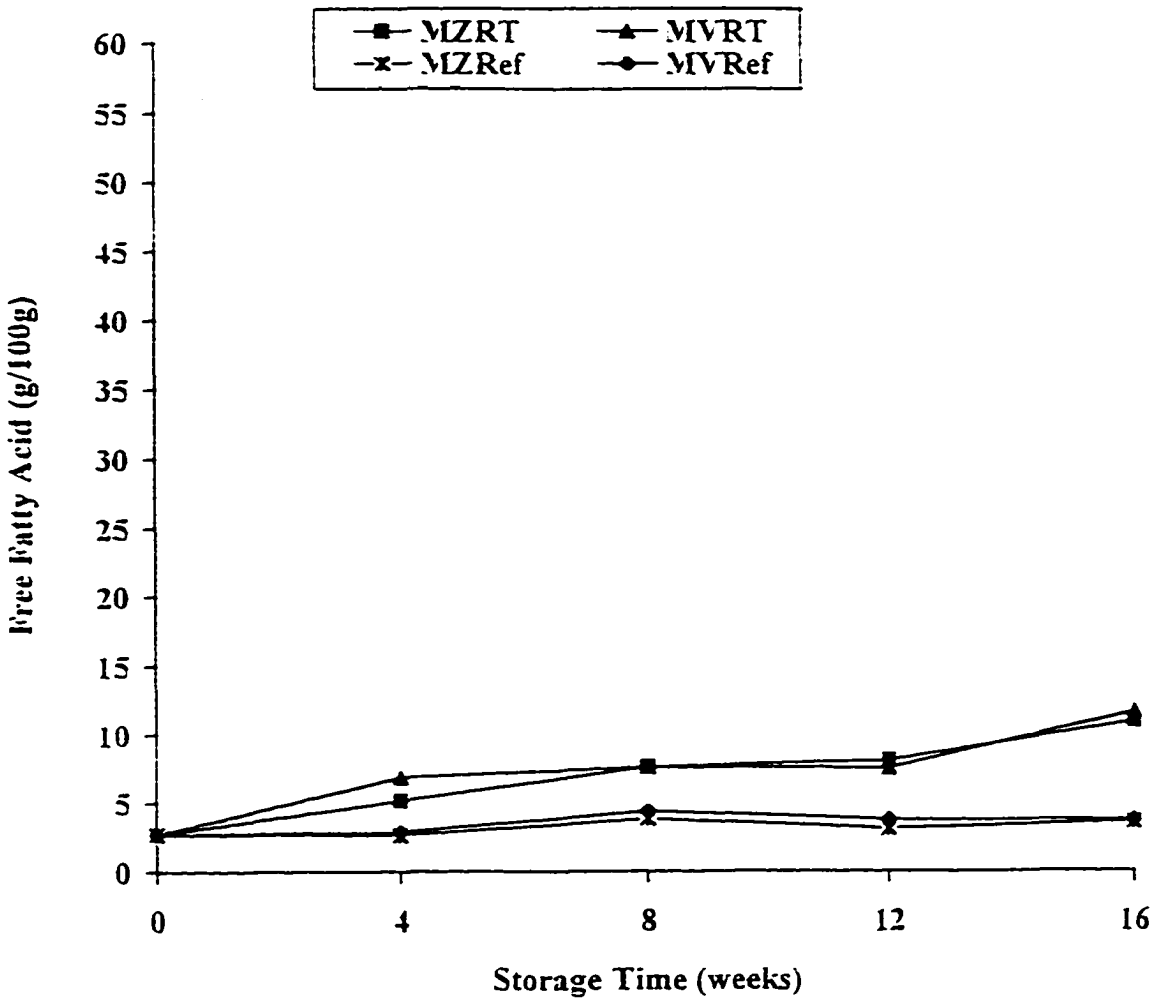


Figure 14. Free fatty acid changes in microwave heat stabilized rice bran during 16 weeks of storage period. Individual values, means and standard deviations presented in Table 20 in the Appendices. M= microwave heat stabilized, Z= Ziplock® bags, V= vacuum packed, RT= room temperature, Ref= refrigerator

As a main effect, packaging method did not show a significant difference between Ziplock® bags and vacuum packing on LOX activity and FFA levels. Interaction of storage time and storage temperature indicated that LOX activity increased as storage time progressed for samples stored at room temperature, while the samples stored in the refrigerator had a decrease, especially at week 12. FFA levels increased (Figure 14) for samples stored in the refrigerator from the initial value of 2.8% to 3.6% and 3.8% for samples packed in Ziplock® bags and vacuum packed, respectively. These changes were not significant ($p < 0.05$). FFA levels increased steadily and very slowly from an initial value of 2.75% to 10.92% and 11.62% for microwave stabilized samples packed in Ziplock® bags and vacuum packed stored at room temperature, respectively. Interaction of storage time and packaging method showed (Figure 14) that LOX activity fluctuated. Samples packed in Ziplock® bags had much higher activity than vacuum packed samples. There was no effect on FFA level. Interaction between storage temperature and packaging indicated that Ziplock® bag and vacuum packing had more effect on samples stored at room temperature than samples stored in the refrigerator. Table 7 shows the changes for FFA and LOX activity for raw and microwave heat stabilized rice bran at 0 and 16 weeks of storage. The results of this study showed that, overall, the samples stored at room temperature had higher FFA levels and LOX activity than samples stored in the refrigerator. The increase in LOX activity could be due to the effect of light and storage temperature. The striking effect of light has been shown (Sowbhagya and Bhattacharya, 1976) in lightly milled, cured, and parboiled rice lipid during storage at room temperature.

Table 7. Lipoxygenase (LOX) activity and free fatty acid (FFA) content of rice bran packed in Ziplock® bags (Z) and vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

			LOX Activity (dA/min)	FFA (%)
Raw(Control)	0 week		0.18 ± 0.00^{b}	2.53 ± 0.2^{f}
	16 weeks	ZRT	0.04 ± 0.01^{c}	48.01 ± 1.0^{b}
		ZRef	0.07 ± 0.00^{c}	19.45 ± 1.8^{d}
		VRT	0.03 ± 0.00^{c}	54.29 ± 0.6^{a}
		VRef	0.07 ± 0.02^{c}	25.37 ± 0.3^{e}
Microwave	0 week		0.18 ± 0.00^{b}	2.75 ± 0.1^{f}
	16 weeks	ZRT	0.54 ± 0.11^{a}	10.93 ± 0.6^{e}
		ZRef	0.08 ± 0.01^{c}	3.58 ± 0.1^{f}
		VRT	0.26 ± 0.10^{b}	11.62 ± 0.0^{e}
		VRef	0.07 ± 0.03^{c}	3.74 ± 0.2^{f}

[a]Means (average of 2 values) within a column with different letters are significantly different ($p < 0.05$).

Oxidative rancidity was much higher in samples placed under light at all times compared to the same samples kept in containers and placed in the dark. Light caused more increase in oxidation rancidity on samples stored at room temperature. Sowbhagya and Bhattacharya (1976) concluded that a relatively high moisture content, storage in dark and low temperature were the important protectants of rice lipids against rancidification.

Microwave heat stabilized samples packed in Ziplock® bags and stored at room temperature had much higher LOX activity than raw rice bran kept under the same conditions. This could be due to the lack or loss of antioxidant present in rice bran samples during microwave heating. As early as 1943, Gyorgy and Tomarelli noticed that brown rice had a beneficial antioxidant activity which was reduced either upon milling or upon autoclaving (120°C, 30 min). Rice bran and rice bran oil contain a good amount of potent antioxidant such as oryzanol, ferulic acid, and esters of unsaturated triterpenoid alcohols (Sowbhagya and Bhattacharya, 1976). These compounds can be lost at the time of milling (Sowbhagya and Bhattacharya, 1976) and/or lose their activity or be destroyed during microwave heating (Yoshida et al., 1991). Microwave heating for various periods of time destroyed α-tocopherol. The loss of activity in tocopherols was found to increase in the order δ-, β-, γ-, and α-tocopherol (the most potent) with microwave heating of the oils. The effectiveness of tocopherols as lipid antioxidants has been attributed to their ability to break chain reactions by reacting with fatty acid peroxy radicals. The findings in this study agree with the above conclusions. As a result, the LOX activity increased. Also, the

increase in LOX activity in microwave heat stabilized rice bran stored at room temperature could be due to an increase in the concentration of transition metals such as copper, cobalt, chromium, and especially iron with an increase in extrusion temperature in the corn starch/soybean oil model system (Rao and Artz, 1989). They reported that most of metals were present at concentrations that were highly catalytic with respect to oxidation (LOX activity). Shastry and Raghavendra Rao (1975) reported that partially purified LOX from unfractionated rice bran of an indica variety was activated by Fe^{++}. Hiroyuki et al. (1986) indicated that rice LOX-3 was inactivated gradually in storage because of participation of metal ions and linoleic hydroperoxide. Champagne et al. (1992) reported that increased susceptibility of ethanol-extracted brown rice kernel to oxidative deterioration could be attributed to ethanolic and heat denaturation of the homoproteins catalase and peroxidase found in the kernel. They suggested unfolding the enzymes to bring about greater exposure of the heme groups to the substrate, allowing the heme group to initiate oxidation. Microwave heat (107 ± 2°C, for 3 min) could be a contributing factor in the increase in LOX activity in microwave heat stabilized samples compared to untreated samples.

The main effect of packaging was not significant ($p > 0.05$) on fatty acids, specifically for linoleic acid, but the main effect of storage temperature was highly significant ($P< 0.001$). The main effect and interactions of factors for proximate composition were not significant ($p > 0.05$). Percent protein and fat did not change significantly in raw (R) and microwave heat (M) stabilized samples packed in Ziplock® bag (Z) and vacuum packed (V) stored at room temperature (RT) and in the

refrigerator (Ref) during 16 weeks of storage (Table 8). Moisture content was significantly different for raw and microwave heated samples at week 0. Raw samples showed (Table 8) a significant increase for ZRef and VRef and a significant decrease for VRT. Microwave heat stabilized samples showed a significant decrease in moisture content for ZRT, ZRef, and VRT, and VRef (Table 8) regardless of packaging methods and storage temperature. The interaction of packaging method and storage temperature was significant ($p < 0.05$) on both fatty acid and proximate composition during storage period.

Conclusions. In this study, raw rice bran was stabilized with microwave heat to deactivate the lipases and LOX, the destructive enzymes. The two different packaging used were Ziplock® bags and vacuum packing of the samples in non-permeable bags. The temperature settings (environmental factor) were ambient temperature (25°C) and refrigerator (4-5°C). The results showed that microwave heat stabilized samples stored at room temperature had an increase of activity in both enzymes. Vacuum packing caused more increase in lipase activity. Lipase and LOX activity was decreased drastically by storing the samples in the refrigerator. Fatty acid and proximate composition did not change significantly ($p > 0.05$) for microwave heat stabilized samples stored in the refrigerator. From data collected it appears that vacuum packing has no advantage over the Ziplock® bag. As a result, the best storage temperature and packaging method to reduce rancidity for microwave heat stabilized rice bran in this study were the Ziplock® bag and refrigeration of the samples over 16 weeks of storage period.

Table 8. Proximate composition of rice bran as affected by packaging and storage temperature[a].

			Protein (%)	Fat (%)	Moisture (%)	Ash (%)
Raw(Control)	0 week		17.1 ± 0.6[a]	16.4 ± 0.0[a]	7.5 ± 0.1[b]	7.4 ± 0.1[bc]
	16 weeks	ZRT	17.4 ± 0.1[a]	16.9 ± 0.0[a]	7.0 ± 0.0[bc]	8.3 ± 0.2[a]
		ZRef	17.0 ± 0.2[a]	17.6 ± 1.1[a]	9.3 ± 0.1[a]	7.9 ± 0.1[ab]
		VRT	17.5 ± 0.2[a]	16.9 ± 0.2[a]	6.5 ± 0.2[c]	8.4 ± 0.2[a]
		VRef	17.0 ± 0.1[a]	16.9 ± 0.2[a]	8.5 ± 0.2[a]	7.8 ± 0.3[bc]
Microwave	0 week		17.5 ± 0.4[a]	17.5 ± 0.4[a]	8.4 ± 0.4[a]	7.6 ± 0.1[bc]
	16 weeks	ZRT	17.7 ± 0.4[a]	17.9 ± 0.3[a]	6.4 ± 0.2[c]	8.4 ± 0.1[a]
		ZRef	17.9 ± 0.3[a]	17.3 ± 0.0[a]	6.3 ± 0.1[c]	8.2 ± 0.1[a]
		VRT	18.0 ± 0.1[a]	17.0 ± 0.1[a]	6.3 ± 0.6[c]	8.4 ± 0.1[a]
		VRef	17.5 ± 0.3[a]	17.1 ± 0.1[a]	7.6 ± 0.7[b]	8.3 ± 0.0[a]

[a]Means (average of 2 values) within a column with different letters are significantly different ($p < 0.05$).
Z = Ziplock® bag, V = vacuum pack, RT = room temperature, Ref = refrigerator.
Carbohydrate content was calculated as 100 - (%Protein + %Fat + %Moisture + %Ash).

CHAPTER 7
SUMMARY AND CONCLUSIONS

Rice bran is a by-product which is produced during the process of milling. The bran constitutes 7-8.5% of the total rice grain. Rice bran is rich in nutrients such as protein, oil, vitamins, oryzanol, and essential minerals. Rice bran also contains enzymes, microorganisms, and insects which could be harmful. To extend the shelf-life of rice bran, some beneficial compounds (essential fatty acids, protein, vitamins) should be preserved, and the harmful compounds (lipase, LOX, microorganisms) must be removed or their activity arrested during processing and storage.

After the bran layer is removed from the endosperm during milling, the individual cells are disrupted, and the rice bran lipids come into contact with a highly reactive lipase enzyme. Freshly milled rice bran has a very short shelf-life because of the decomposition of lipids into FFA and glycerol (hydrolytic rancidity), making it unsuitable for human consumption and the economical extraction of edible oil. The FFA goes under further decomposition (oxidative rancidity) and results in not only free radicals but also bad taste and loss of nutritional values. However, if the bran is subjected to a short-term high-temperature heat treatment immediately after milling, the lipase activity is deactivated and stable bran is produced.

In the present study, microwave heat was used to stabilize the rice bran. Rice variety 'Lemont', cultivated at the Louisiana Rice Experiment Station, Crowley, Louisiana was used. Freshly milled raw rice bran (150 gram per batch) was exposed to microwave heat for 3 min. The moisture content of the sample was adjusted from 7.7% to 21%. Half of the raw bran (control) and stabilized samples were vacuum

packed in non-permeable polyethylene bags, and the other half was packed in polyethylene Ziplock® bags. Half of the samples were stored in the refrigerator (5°C) and the other half were stored at room temperature (25°C). For testing the efficiency of treatment and effect of microwave heat, the samples were analyzed for free fatty acid (FFA), lipoxygenase activity, specific activity, and fatty acid composition after 0, 4, 8, 12, and 16 weeks of storage. Samples were analyzed for proximate composition at week 0 and 16.

Results obtained on rice bran from the effect of microwave heat on bran stability in terms of increase in FFA (% oleic acid) were analyzed statistically and studied. FFA level increased significantly ($p < 0.05$) in raw rice bran samples stored at room temperature from an initial value of 2.5% to 48.0% and 54.3% in samples packed in Ziplock® bags and vacuum packed, respectively. FFA level in raw rice bran samples stored in the refrigerator increased from an initial value of 2.5% to 19.5% and 25.4% in samples stored in the refrigerator and packed in Ziplock® bags and vacuum packed, respectively. Microwave heat stabilized samples had a significant ($p < 0.05$) increase in FFA level in samples stored at room temperature and packed in Ziplock® bags and vacuum packed with an initial value of 2.8% to 10.9% and 11.6%, respectively. However, the FFA level in microwave heat stabilized samples stored in the refrigerator did not increase significantly ($p > 0.05$) during 16 weeks of storage. Data collected show that vacuum packing did not show any advantage over Ziplock® bags, and the best temperature for storage of microwave heat stabilized rice bran was refrigeration. LOX activity in raw rice bran samples decreased significantly ($p < 0.05$)

from initial value of 0.18 dA/min to 0.04 and 0.03 dA/min for samples stored at room temperature and packed in Ziplock® bags and vacuum packed, respectively, during storage. LOX activity was reduced from an initial value of 0.18 dA/min to 0.07 and 0.07 dA/min for samples stored in the refrigerator in two types of packaging.

Microwave heat stabilized samples stored at room temperature in Ziplock® bag and vacuum packed had a significant ($p < 0.05$) increase in LOX activity from an initial value of 0.18 dA/min to 0.54 and 0.26 dA/min, respectively during storage period. Samples stored in Ziplock® bags showed a higher increase. However, LOX activity in microwave heat stabilized samples stored at 4-5°C decreased significantly from initial values of 0.18 dA/min to 0.07 and 0.07 dA/min regardless of packaging. From data obtained, it can be concluded that storage temperature of 4-5°C (refrigerator) and Ziplock® packaging was best for controlling LOX activity in microwave heat stabilized samples. Further studies are recommended to study the effect of microwave heat, packaging methods, and storage temperature on autoxidation of lipids in rice bran.

Fatty acid composition, specifically linoleic acid, did not change significantly ($p > 0.05$) from an initial value of 32.6% to 30.3% and 30.4% in microwave heat stabilized rice bran samples packed in Ziplock® bags and vacuum packed and stored in the refrigerator (4-5°C) during 16 weeks of storage. Proximate composition of microwave heat stabilized rice bran samples did change significantly ($p > 0.05$) for samples packed in Ziplock® bags and vacuum packed and stored in the refrigerator during storage. The moisture content decreased significantly ($p< 0.05$) from the initial

value of 8.4% to 6.3% and 7.6% for samples stored in the refrigerator, packed in Ziplock® bags and vacuum packed, respectively. Data collected in this experiment indicate that use of vacuum packing does not have any advantage over Ziplock® bags for storage, and the refrigerator (4-5°C) is the suitable temperature to extend the shelf-life in microwave heat stabilized rice bran samples during 16 weeks of storage.

Recommendations. It is recommended that further studies be made:

1. To incorporate microwave heat stabilized rice bran in products such as breads, muffins, meatballs, cookies, etc. and perform sensory evaluations with highly trained panelists of the new products and compare these to the products developed with raw rice bran (control) and regular flour using exactly the same recipe.
2. To study the effect of storage temperature and packing materials and methods for longer period of time (6 months to 1 year).
3. To determine vitamin E and oryzanol (antioxidant) in microwave heat stabilized vs. control samples and study the changes in two different packaging methods and two different storage temperature.
4. To determine the specific oxidation products from LOX activity and autoxidation in microwave heat stabilized samples and study the effect of packaging methods and storage temperature during storage.

REFERENCES

Aizono, Y., Fujiki, Y., and Funatsu, M. **1976**. Purification of rice bran lipase and its multiple form. Protein, nucleic acid and enzyme. Kyoritsu Shuppan Co., Tokyo. 76(2):203.

Aizono, Y., Funatsu, M., Hayashi, K., Inamasu, M., and Yamaguchi, M. **1971**. Biochemical studies of rice bran lipase. Part II. Chemical properties. Agricultural Biological Chemistry. 35(12):1973 1979.

Andre, J.C., and Funk, M.O. **1986**. Determination of stereochemistry in the fatty acid hydroperoxide products of lipoxygenase catalysis. Analytical Biochemistry. 158:316-321.

AOAC. **1991**. Official Method of Analysis. S. Williamss., (ed). 15th ed. AOAC publ. Association of Official Analytical Chemists. Washington, D.C.

AOCS. **1991**. Fatty acids composition by GLC in marine oils. Association of Oil Chemist Society. Official method Ce1b-89.

Aurand, L.W., Woods, A.E., and Wells, M.R. **1987**. Enzymes in: Food Composition and Analysis. An AVI book. AVI Publishing Co., Westport, NY. pp 283-348.

Barnes, P. and Galliard, T. **1991**. Rancidity in cereal products. Lipid Technology. 3:23-28.

Ben-Aziz, A., Grossman, S., Ascarelli, I., and Budowski, P. **1970**. Linoleate oxidation induced by lipoxygenase and heme proteins: a direct spectrophotometric assay. Analytical Biochemistry. 34:88-100.

Berry, H., Debat, H., and Larreta-Garde, V. **1977**. Excess substrate inhibition of soybean lipoxygenase-1 is mainly oxygen-dependent. FEBS letters. 408:324-326.

Boyes, S., Peyera, C., and Young, H. **1992**. Kiwifruit lipoxygenase: preparation and characteristics. Journal of Food Science. 57(6):1390-1394.

Brown, B. D., Wei, L.S., Steinberg, M.P., and Villota, A. **1982**. Minimizing protein insolubilization during thermal inactivation of lipoxygenase in soybean cotyledones. Journal of American Oil Chemists Society. 59(2): 88-92.

Champagne, E.T. and Hron, R.J. Sr. **1992**. Stabilizing brown rice to lipolytic hydrolysis by ethanol vapors. Cereal Chemistry. 69:152-156.

Champagne, E.T., Hron, R.J. Sr., and Abraham, G. **1992**. Utilizing ethanol to produce stabilized brown rice products. Journal of American Oil Chemists Society. 69:205-208.

Copper, T. G. **1942**. Protein isolation and purification. In: The Tools of Biochemistry. University of Pittsburgh. John Wiley and Sons, New York. pp 355-405.

Dai-Eun, S. and MeeRee, K. **1989**. The possible role of 9(S)-hydroperoxyoctadecatrienoic acid as a suicide substrate of soybean lipoxygenase. Biochemical and Biophysical Research Communications. 162(3):1357-1362.

DeGroot, J. J. M. C., Veldink, G. A., Vliegenthart, J. F. G., Boldingh, J., Waver, R., and VanGelder, B. F. **1975b**. Demonstration by EPR spectroscopy of the functional role of iron in soybean lipoxygenase. Biochem. Biophys. Acta, 337: 71-79.

Desikachar, H. S. **1974**. Status report: Prevention of by-products of rice milling. In: Proc. Rice By-products utilization int. conf. II. pp 1.

Dhaliwal, Y.S., Sekhon, K.S., and Nagi, H.P.S. 1991. Enzymatic activities and rheological properties of stored rice. Cereal Chemistry. 68(1):18-21.

Dixon, M. and Webb, E.C. **1961**. Enzyme fractionation by salting-out: a theoretical note. In: Advances in Protein chemistry, Vol. 16. Anfinsen, C.B. et al.(ed). Academic Press, N Y. pp 197-219.

Enochian, R. V., Saunders, R. M., Schultz, W. G., Beagle, E. C., and Crowley, P. R. **1980**. Stabilization of rice bran with extrusion cookers and recovery of edible oil: a preliminary analysis of operation and financial feasibility. Marketing Research Report 1120. ARS. USDA. Western Regional Research Center, Barkely, CA.

Esaka, M., Suzuki, K., and Kubota, K. **1986**. Inactivation of lipoxygenase and trypsin inhibitor in soybean on microwave irridiation. Agricultural and Biological Chemistry. 50:2395.

Esaka, M., Suzuki, K., and Kubota, K. **1987**. Effect of microwave heating on lipoxygenase and trypsin inhibitor activities, and water absorption of winged bean seed. Journal of Food Science. 52(6):1738-1739.

Frankel, E. N. **1982**. Volatile lipid oxidation products. Prog. Lipid Research. 22:1.

Frankel, E. N. **1984.** Lipid oxidation: Mechanisms, products and biological significance. Journal of American oil Chemists Society. 61(12): 1908-1916.

Galliard, T. and Chan, H.W.S. **1980.** The Biochemistry of Plants, Vol. 4. Stump, P.K.(ed). Academic Press Inc., N Y. pp 131.

Ganthavorn, C., Nagel, C.W., and Powers, J.R. **1991.** Thermal inactivation of asparagus lipoxygenase and peroxidase. Journal of Food Science. 56 (1):47-49.

Gardner, H. W. **1979.** Lipid enzymes: lipases, lipoxygenase and "HYDROPEROXIDASES" In: Autooxidation in Food and Biological Systems. Simis, M.G. and Karel, M. (eds). Plenum Press. New York and London, pp 447-504.

Gardner, H. W. **1988.** Lipoxygenase pathway in cereals. In: Advances in Cereal Sciences and Technology,. Pomeranz Y. ed. Volume IX, American Association of Cereal Chemists, St. Paul, MN, pp 161-215.

Gibian, M.J. and Vandenberg, P. **1987.** Product yield in oxygenation of linoleate by soybean lipoxygenase: The value of the molar extinction coefficient in the spectrophotometric assay. Analytical Biological Chemistry. 163: 343-349.

Graveland, A. **1970.** Enzymatic oxidation of linoleic acid and glycerol-1-monolinoleate in dough and flour - water suspension. Journal of American Oil Chemists Society. 47:352-361.

Grosch, W. **1987.** Reactions of Hydroperoxides, products of low molecular weight. In: Autoxidation of unsaturated lipids. Chan, H. W. S (ed). Academic Press, New York and London. pp. 95-139.

Gupta, H. **1989.** Rice bran offers India an oil source. Journal of American Oil Chemists Society. 66(5):620-623.

Gyrogy, P. and Tomarelli, R. **1943.** Antioxident activity in sources of the B vitamins. Journal of Biological Chemistry. 147:515-519.

Hafez, Y.S., Mohamed, A.I., Hewedy, F.M., and Singh,G. **1985a.** Effect of microwave heating on soluability, digestibility and metabolism of soy protein. Journal of Food Science. 50:415-417.

Hafez, Y. S., Mohamed, A. I., Sigh, G., and Hewedy, F. M. **1985b.** Effect of gamma irradiation on proteins and fatty acids of soybean. Journal of Food Science. 50:1271-1274.

Hamberg, M. and Samuelsson, B., **1967**. On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxidase. Journal of Biological Chemistry. 242: 5329.

Hegsted, M., Windhauser, M.M., Lester, S.B., and Morris, S.K., **1993**. Stabilized rice bran and oat bran lower cholesterol in human. Nutrition Research. 13:387-398.

Henderson, S.M. and Perry, R.L. **1976**. Agricultural Process Engineering. 3rd ed. An AVI book , Westport, CT.

Hiroyuki, O., Shoji, I., Bunzo, M., and Yuhei, M. **1986**. Purification and characterization of rice lipoxygenase component 3 from embryos. Journal of Agricultural and Biological Chemistry. 50(120:3165-3171.

Hoffpauir, C.L., Petty, D., and Guthrie, J.D. **1947**. Germination and free fatty acids in individual cottonseeds. Science 106: 344-345.

Houston, D.F. **1972**. Rice bran and polish. In: Rice Chemistry and Technology. Houston, D.F. (ed),. Amer. Assoc. of Cereal Chemist. St. Paul pp1-56

Ida, S., Masaki, Y., and Morita, Y. **1983**. The isolation of multiple forms and product specificity of rice lipoxygenase. Agricultural Biological Chemistry. 47(3):637-641.

Kahlon, T.S., Saunders, R.M., Chow, F.I., Chui, M.M., and Betschart, A.A. **1990**. Influence of rice bran, oat bran, and wheat bran on cholesterol and triglycerides in hamster. Cereal Chemistry. 67:439-443.

Kahlon,T.S., Chow, F.I., Sayre, R.N., and Betschart, A.A. **1991**. Cholesterol-lowering in hamsters fed rice bran at various levels, defatted rice bran and rice bran oil. American Institute of Nutrition. pp 513-519.

Klein, L.B. and Mondy, N.I. **1981**. Comparison of microwave and conventional baked potatoes in relation to nitrogenous constituents and mineral composition. Journal of Food Science. 46:1874-1879.

Koch, R.B. **1961**. Dehydrated foods and model systems. In:Symposium on Foods, lipids and their oxidation. Schulz, H.W. (ed). An AVI Book, Westport, CT.

Kishnamurthy, Nasirullah M. N. and Nagaraja, K.V. **1989**. Effect of stabilization on the quality characteristics of rice bran oil. Journal of American Oil Chemists Society. 66(5):661-663.

Labuza, T.P. **1977**. Fats, fatty acids, and cholesterol. In:Demons the table spread? Labuza, T.P. (ed). An AVI Book, Westport, CT. pp 67-79.

Laning, S.J. **1991**. Fats, oil, fatty acids, and oil seed crops. In:Biotechnology and Food Ingredients. Goldberg, I., and Williams, R.(eds). An AVI Book, Westport, CT. pp 265-313.

Layne, E. **1957**. Spectrophotometric and turbidimetric methods for measuring Proteins, In: Methods in Enzymology, Colowick, S.P. and N.O. Kaplan (eds). Vol.3. Academic Press, NY. p. 447.

Lee, F.A.1975. Water and solutions. In: Basic Food Chemistry. Lee, F.A. (ed). An AVI Book, Westport, CT. pp 9-24.

Lehmann, W. D., Metzger, K., Stephan, M., Wittig, U., Zalan, I., Habenicht, A. J., and Furstenberger, G. **1995**. Quantitative lipoxygenase product profiling by gas chromatography and negative ion chemical ionization mass spectrometry. Analytical Biochemistry. 224(1):227-234.

Lehmann, W. D., Stephan, M., and Furstenberger, G. **1992**. Profiling assay for lipoxygenase products of linoleic acid and arachidonic acid by gas chromatography-mass spectrometry. Analytical Biochemistry. (204):1. pp 158-170.

Lin, S. h. C., and Carter, C. M. **1973**. Effect of extrusion cooking on the formation of free fatty acids in rice bran. Food Protein R&D Center. Texas A&M University College Station.

Lineweaver, H. and Burk, D. **1934**. The determination of enzyme dissociation constants. Journal of American Chemists Society. 56:686.

Loeb, J. R., Morris, N. J., and Dollear, F. G. **1949**. Rice bran oil IV. Storage of the bran as it affects hydrolysis of the oil. Journal of American Oil Chemists Society. 26: 738-743.

Lu, S. and Luh, B.S. **1991**. Properties of the rice caryopsis. In: Rice Production. 2nd ed. Vol. 1. Luh, B. S. (ed). An AVI Book. Westport, CT. pp 389-419.

Malekian, F. **1992**. Functional, nutritional and storage characteristics of rice bran as affected by microwave heat and extrusion stabilization methods. Thesis, Louisiana State University, Baton Rouge.

Marci, F., Braidot, E., and Vianello, A. **1994**. Lipoxygenase activity associated to isolated soybean plasma membranes. Biochem. Biophys Acta. 215(1-2):109-114.

Martin, D.E., Godber, J.S., Setlhako, G., Verma, L.R., and Wells, J.H. **1993**. Optimizing rice bran stabilization by extrusion cooking. Louisiana Agriculture. 36(3):13-15.

Martin, D.E., Verma, L.R., Godber, J.S., and Wells, J.H. **1991**. Extrusion processing of rice bran. ASAE paper No 916028. Presented at the international summer meeting sponsored by American Society of Agricultural Engineering. Albuquerque, New Mexico.

Mattson, F.H. and Grundy, S.M. **1985**. Comparison effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. Journal of Lipid Research. 26:194-199.

McDonald, B.E., Gerrard, J.M., Bruce, V.M., and Corner, E.J. **1989**. Comparison of the effect of canola oil and sunflower oil on plasma lipids and lipoproteins and on in vivo thromboxane A_2 and prostacyclin production in healthy young men. American Journal of Clinical Nutrition. 50:1282-1286.

Michal, S. **1978**. Determination of Michaelis constant and inhibitor constant. In: Principles of Enzymatic Analysis. Bergmeyer, H.U.(ed). 1st edition, Weinhim, NY. pp 29-40.

Mikkelsen, D.S., and de Datta, S.K. **1991**. Rice culture. In: Rice Production. 2nd ed. Vol. 1. Luh, B.S. (ed). AVI Publishing Co., Westport, CT. pp 103-120.

Mitsuda, H.F., Kawai, F., and Yamamoto, A. **1972**. Underwater and undeground storage of cereal grains. Food Technology. 26(3):50-54.

Mitsuda, H.F., Yasumoto, K., and Yamamoto, A. **1967a**. Inactivation of lipoxygenase by hydrogen peroxide, cysteine and some other reagents. Agricultural and Biological Chemistry. 31:853-860.

Mitsuda, H.F., Yasumoto, K., Yamamoto, A., and Kusano, T. **1967b**. Part 1. Preparation of crystalline enzyme and assay by polarographic method. Agricultural Biological Chemistry. 31(1):115-118.

Nawar, W.W. **1985**. Lipids. In: Food Chemistry. Fennema, O.R. (ed). 2nd ed. Marcel Dekker Inc. New York NY. pp 139-244.

Newman, R.K., Betschart, A.A., Newman, C.W., and Hofer, P.J. **1992**. Effect of full-fat or defatted rice bran on serum cholesterol. Plant Foods for Human Nutrition. 42:37-43.

Nicolosi, R.J., Ausman, L.M., and Hegsted, D.M. **1991**. Rice bran oil lowers serum total and low density lipoprotein cholesterol and apo B levels in nonhuman primates. Atheroscerosis. 88:133-142.

O'Connor, T. P. and O'Brien, N. M. **1991**. Significance of lipoxygenase in fruits and vegetables. In: Food Enzymology. Fox, P. F. (ed). Elsevier Science publishing Co., Inc. New York, NY. pp 338-364.

Piggott, J.R., Morris, W.R., and Clyne, J. **1991**. Changes in lipids and in sensory attributes on storage of rice milled to different degree. International Journal of Food Science and Technology. 26:615-628.

Prabhakar, J. V., and Venktesh, K. V. L. **1986**. A simple chemical method for stabilization of rice bran. Journal of American Oil Chemists Society. 63:644-646.

Price, N.C. and Stevens, L. **1991**. Enzyme: structure and function. In: Food Enzymology. Fox, P.F. (ed). Volume 1. Elsevier Science Publishing Co., NY pp 1-52.

Ramarathnam, N., Osawa, T., Namiki, M., and Kawakishi, N. **1989**. Studies on changes in fatty acid composition and content of endogenous antioxidant during γ irradiation of rice seeds. Journal of American Oil Chemists Society. 66(1):105-108.

Rao, S.K. and Artz, W.E. **1989**. Effects of extrusion on lipid oxidation. Journal of Food Science. 54:1580-1583.

Richardson, T. and Hyslop, D.B. **1985**. Enzymes in : Food Chemistry. Fennema, O.R. (ed). Marcel Dekker Inc., New York NY 2nd ed. p:447.

Royo, J., Vancanney, T. G., Perez, A. G., Sanz, C., Stormann, K., Rosahl, S., and Sanchez-Serrano, J. J. **1996**. Characterization of three potato lipoxygenase with distinct enzymatic activities and different organ-specific and wound-regulated expression patterns. Journal of Biological Chemistry. 271(35):21012-21019.

Rukmini, C. and Raghuram, T.C. **1991**. Nutritional and biochemical aspect of the hypolipidemic action of rice bran oil: a review. Journal of the American College of Nutrition. 10(6):593-601.

SAS. **1997**. SAS/STAT Users Guide. Version 6.12. SAS Institute, Inc,. Cary, NC.

Saunders, R.M. **1985**. Rice bran: composition and potential food sources. Food Review International. 1(3):465-495.

Saunders, R.M. **1990**. The properties of rice bran as a food stuff. Cereal Foods World. 35(7): 632-636.

Sayre, R.N., Saunders, R.M., Enochian, R.V., Schultz, W.G., and Beagle, E.C. **1982**. Review of rice bran stabilization systems with emphasis on extrusion cooking. Cereal Foods World. 27(7):317-322.

Seetharamaiah, G.S. and Chandrasekhara, N. **1989**. Studies on hypocholesterolemic activity of rice bran oil. Atherosclerosis. 78:219-223.

Sekhar, B.P.S. and Reddy, G.M. **1982**. Studies on lipoxygenase from rice (*Oryza sativa L.*). Journal of Science and Food Agriculture. 33:1160-1163.

Sharp, R.N. and Timme, L.K. **1986**. Effect of storage time, storage temperature, and packing methods on shelf-life of brown rice. Cereal Chemistry. 63(3):247-251.

Shastry, B.S. and Raghavendra Rao, M. R. **1975**. Studies on lipoxygenase from rice bran. Cereal Chemistry. 52(5):597-603.

Shin, M. G., Yoon, S. H., Rhee, J. S., and Kwon, T. **1986**. Correlation between oxidative deterioration of unsaturated lipid and n-hexanal during storage of brown rice. Journal of Food Science. 51(2):460-463.

Skrzypezak-Jankun, E., Amzel, L. M., Kroa, B. A., and Funk, M. O.,Jr. **1997**. Structure of soybean lipoxygenase L3 and a comparison with its L1 isoenzyme. Protein. 29(1):15-31.

Sowbhagya, C.M. and Bhattacharya, K.R. **1976**. Lipid oxidation in rice. Journal of Food Science. 41:1018-1023.

St.Angelo, A. J., Ory, R. L., and Brown, L. E. **1972**. A comparison of minor constituents in peanut butter as possible source of fatty acid peroxidation. J. American Peanut Research Education Association. 4:186-197.

Surrey, K. **1964**. Spectrophotometric method for determination of lipoxidase activity. Plant Physiology. 39:65-70.

Tai, C.C., Li, K.H., Guo, M.S., Li, C.F., Chao, R.S., and Chen, S.C. 1981. Storage of milled rice in plastic bags flushed with carbon dioxide in vacuum. Food Ind. Dev. Inst., Res. Rep. No. E-18. (In Food Science and Technology. Abstract. 1983. 15(1):1M41).

Takahama, U. **1985**. Inhibitation of lipoxygenase-dependent lipid peroxidation by quercetin: mechanism of antioxidative function. Phytochemistry, 24(7):1443-1446.

Takano, K. **1993**. Mechanism of lipid hydrolysis in rice bran. Cereal Foods World. 38 (9):695-698.

Tao, J. **1989**. Rice bran stabilization by improved internal and external heating methods. Ph.D. Dissertation, Louisiana State University, Baton Rouge.

Tao, J., Rao, R.M., and Liuzzo, J.A. **1993**. Thermal efficiencies of conventional and microwave heat stabilization of rice bran. Louisiana Agriculture. 36(3):15.

Tappel, A.L. **1963**. Lipoxidase. In: Enzymes. Boyer, P.D., Lardy H., and Myrback, K.(eds).; 2nd ed. Academic Press : New York. vol B. p 275.

Theorell, H., Holman, R. T., and Akeson, A, **1947**. A note on the preparation of crystalline soybean lipoxidase. Arch. Biochemistry. Biophysics. 14:250-252.

Tomlin, J. and Read, N.W. **1988**. Comparison of the effects on colonic function caused by feeding rice bran and wheat bran. European Journal of Clinical Nutrition. 42:857-861.

Tsen, C.C., Reddy, P.R.K., and Gehrke, C.W. **1977**. Effects of conventional baking, microwave baking, and steaming on the nutritive value of regular and fortified breads. Journal of Food Science. 42(2):402-406.

Urbanski, A.L. **1990**. The rice bran comment. Food and Beverage Marketing. p.71.

Wadsworth, J.I. and Koltun, S.P. **1986**. Physiochemical properties and cooking quality of microwave dried rice. Journal of Agriculture and Food Chemistry. 39:1860-1862.

Wallace, J.M. and Wheeler, E.L. **1972**. Lipoxygenase inactivation in wheat protein concentrate by heat-moisture treatments. Food Chemistry. 49:92-97.

Wang, S. H. and Toledo, M. C. F. **1987**. Inactivation of soybean lipoxygenase by microwave heating: effect of moisture content and exposure time. Journal of Food Science. 52(5):1344-1347.

Whitaker, J. R. **1991**. Lipoxygenase. In: Oxidative Enzymes in Foods. Robenson, D. S. and Eskin, N. A. M. (eds). Elsevier Science Publishing Co., Inc. NY. pp 175-207.

Williams, D. C., Lim, M. H., Chen, A. O., Pangborn, R. M., and Whitaker, J. R. **1986**. Blanching of vegetables for freezing- which indicator enzyme to use. Food Technology. 40 (6):130.

Wu, M.T. **1977**. Extending shelf-life of fresh soybean curds by in-package microwave treatments. Journal of Food Science. 42(6):1448-1450.

Yamamoto, A., Fujii, Y., Yasumoto, K., and Mitsuda, H. **1980**. Partial purification and study of some rice germ lipoxygenase. Agricultural and Biological Chemistry. 44(2):443-445.

Yamamoto, A., Yasumoto, K., and Mitsuda, H. **1970**. Isolation of lipoxygenase isozymes and comparison of their properties. Agricultural and Biological Chemistry. 34(8):1169-1177.

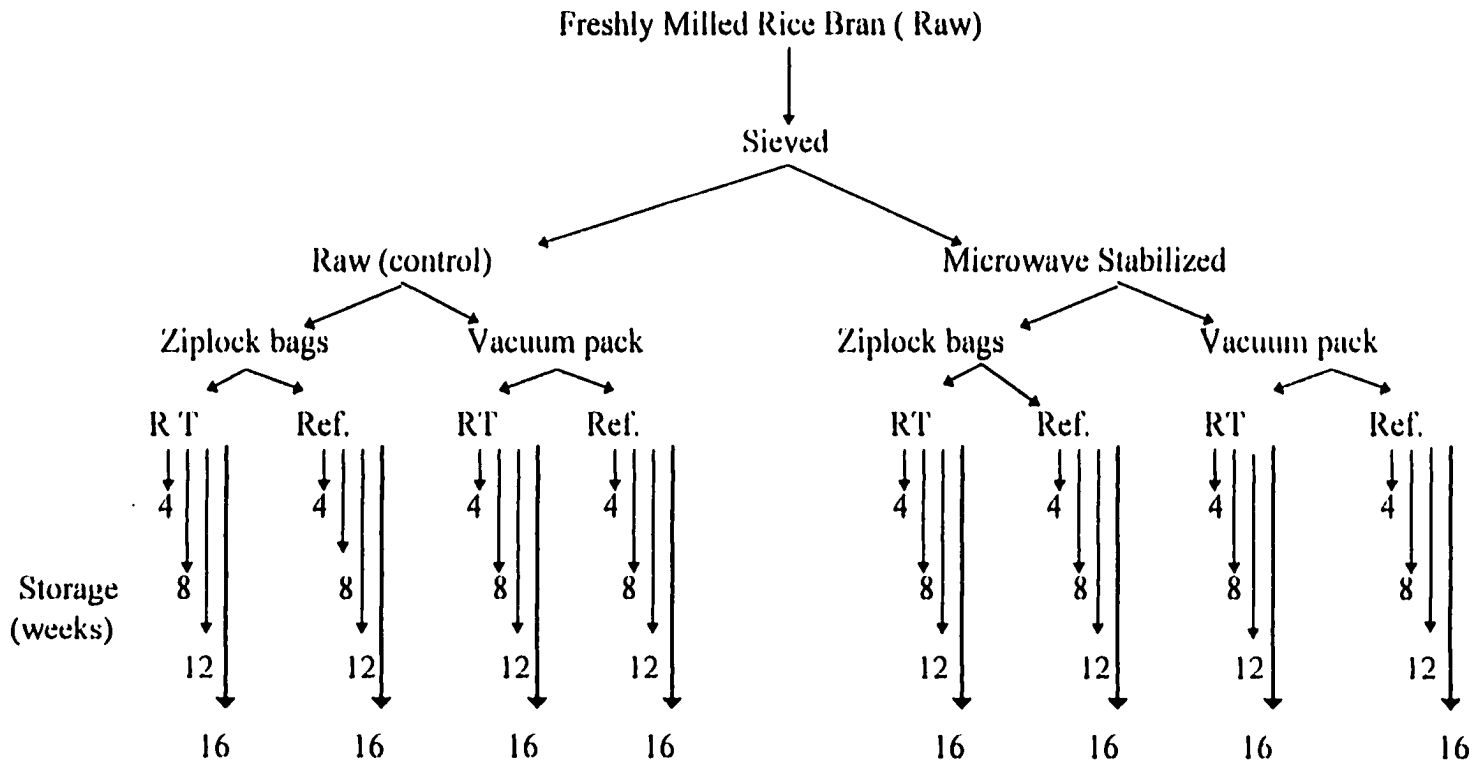
Yeo, H. and Shibamoto, T. **1991**. Effect of moisture content on the Millard browning model system upon microwave irradiation. Journal of Agricultural and Food Chemistry. 39:1860-1862.

Yoshida, H., Hirooka, N., and Kajimoto, G. **1991**. Microwave heating effect on relativestability of tocopherols in oils. Journal of Food Science. 56(4):1042-1046.

Yoshida, H. and Kajimoto, G. **1989**. Effects of microwave energy on the tocopherols of soybean seeds. Journal of Food Science. 54:1596-1600

APPENDICES

Figure 15. Schematic diagram of rice bran processing and storage.



RT: Room Temperature (25°C)
Ref: Refrigerator (3-5°C)

Table 9. Type III sum of squares (SS's) and associated *p*-values for percent free fatty acid. The design of study was 2x2x2x4 factor factorial**.

Source	Degree of Freedom	Type III Sum Square	*P*-value
FactorA	1	9147.72691406	0.0001
FactorB	1	96.35876406	0.0001
FactorA*FatorB	1	64.90316406	0.0001
FactorC	1	4129.50825156	0.0001
FactorA*FactorC	1	2074.91637656	0.0001
FactorB*FactorC	1	4.17691406	0.0213
FactorA*FactorB*FactorC	1	3.75875156	0.0283
week	3	582.18954219	0.0001
FactorA*week	3	241.54430469	0.0001
FactorB*week	3	9.79987969	0.0088
FactorA*Factor B*week	3	13.40712969	0.0018
FactorC*week	3	23.86089219	0.0001
FactorA*FactorC*week	3	7.02496719	0.0331
FactorB*FeatorC*week	3	8.04440469	0.0202
FactorA*FactorB*FactorC*week	3	3.79541719	0.1715

The means (average of 2 values) were significantly different at $p < 0.05$ except for interaction of factors A, B, C, and week.

** Factor A = treatment (raw and microwave heat stabilized) .
Factor B = packaging methods (Ziplock® bags and vacuum packing).
Factor C = storage temperature (25°C and 4-5°C).
Week = storage period of 4, 8, 12, and 16 weeks.

Table 10. Student-Newman-Keuls (SNK) pairwise comparison test for percent free fatty acid (FFA) in rice bran at week 0 and week 16[a].

			Free Fatty acid	SNK
			Mean (%)	Grouping
Raw	0 week		2.5	F
	16 weeks	ZRT	48.0	B
		VRT	54.3	A
		ZRef	19.5	D
		VRef	25.4	C
Microwave	0 week		2.8	F
	16 weeks	ZRT	10.9	E
		VRT	11.6	E
		ZRef	3.6	F
		VRef	3.7	F

[a]Means (average of 2 values) with the same letter are not significantly different ($p < 0.05$).
Z = Ziplock® bag, V = vacuum pack, RT = room temperature (25°C), Ref = refrigerator (4-5°C)

Table 11. Type III sum of squares (SS's) and associated *p*-values for lipoxygenase activity. The design of study was 2x2x2x4 factor factorial**.

Source	Degree of Freedom	Type III Sum Square	*P*-value
FactorA	1	127265.21130625	0.2946
FactorB	1	2208567.51562499	0.0001
FactorA*FatorB	1	627561.03515625	0.0242
FactorC	1	399575.69440001	0.0681
FactorA*FactorC	1	10830.04455625	0.7579
FactorB*FactorC	1	83581.70102500	0.3943
FactorA*FactorB*FactorC	1	219354.06425625	0.1715
week	3	4741063.21578751	0.0001
FactorA*week	3	2993884.12888125	0.0002
FactorB*week	3	1441920.94496249	0.0119
FactorA*Factor B*week	3	133810.70218125	0.2946
FactorC*week	3	330420.15843750	0.4133
FactorA*FactorC*week	3	342764.96603126	0.3970
FactorB*FcatorC*week	3	1545259.29111250	0.0087
FactorA*FactorB*FactorC*week	3	94054.63218125	0.8396

The means (average of 2 values) were significantly different at $p < 0.05$ except for interaction of factors A, B, C, and week.

** Factor A = treatment (raw and microwave heat stabilized) .
Factor B = packaging methods (Ziplock® bags and vacuum packing).
Factor C = storage temperature (25°C and 4-5°C).
Week = storage period of 4, 8, 12, and 16 weeks.

Table 12. Student-Newman-Keuls (SNK) pairwise comparison test for lipoxygenase activity (LOX) in rice bran at week 0 and week 16[a].

			Lipoxygenase Activity	SNK
			dA/min	Grouping
Raw	0 week		0.18	B
	16 weeks	ZRT	0.04	B
		VRT	0.07	B
		ZRef	0.03	B
		VRef	0.07	B
Microwave	0 week		0.18	B
	16 weeks	ZRT	0.54	A
		VRT	0.08	B
		ZRef	0.26	B
		VRef	0.07	B

[a]Means (average of 2 values) with the same letter are not significantly different ($p < 0.05$).
Z = Ziplock® bag, V = vacuum pack, RT = room temperature (25°C), Ref = refrigerator (4-5°C)

Table 13. Free fatty acid means and standard deviations for each point related to Figure 4. Free fatty acid changes in raw (R) and microwave (M) heat stabilized rice bran vacuum packed (V) and packed in Ziplock® bags stored at room temperature (RT) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
		Free Fatty Acid (%)				
Raw(Control)		2.53±0.2				
	ZRT		34.38±0.9	38.61±3.6	41.99±0.0	48.01±0.1
	VRT		38.79±0.2	46.87±1.25	44.89±0.3	54.29±0.6
Microwave		2.75±0.1				
	ZRT		5.15±0.4	7.610.5	8.10±0.0	10.92±0.6
	VRT		5.88±0.1	7.64±0.5	7.52±0.3	11.62±0.0

[a]Means are average of 2 values.

Table 14. Free fatty acid means and standard deviations for each point related to Figure 5. Free fatty acid changes in raw (R) and Microwave (M) heat stabilized rice bran packed in Ziplock® bags (Z) and vacuum packed (V) stored in the refrigerator (Ref) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
				Free Fatty Acid (%)		
Raw(Control)		2.53±0.2				
	ZRef		8.85±0.7	13.42±0.7	15.44±0.1	19.45±1.8
	VRef		9.27±0.1	17.39±0.8	19.02±0.2	25.37±0.3
Microwave		2.75±0.1				
	ZRef		2.65±0.2	3.85±0.7	3.11±0.2	3.58±0.1
	VRef		2.89±0.1	4.42±0.1	3.79±0.1	3.74±0.2

[a]Means are average of 2 values.

Table 15. Lipoxygenase (LOX) activity means and standard deviations for each point related to Figure 9. LOX activity of raw (R) and microwave (M) heat stabilized rice bran packed in Ziplock® bags (Z) or vacuum packed (V) stored at room temperature (RT) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
Raw(Control)		0.18±0.0				
	ZRT		0.21±0.04	0.13±0.04	0.21±0.04	0.04±0.02
	VRT		0.20±0.03	0.21±.05	0.20±0.04	0.03±0.00
Microwave		0.18±0.0				
	ZRT		0.37±0.2	0.23±0.04	0.37±0.07	0.54±0.11
	VRT		0.22±0.05	0.20±0.00	0.22±0.06	0.26±0.16

[a]Means are average of 2 values.

Table 16. Lipoxygenase (LOX) activity means and standard deviations for each point related to Figure 10. LOX activity changes in raw (R) and microwave (M) heat stabilized rice bran packed in Ziplock® bags (Z) or vacuum packed (V) stored the refrigerator (Ref) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
Raw(Control)		0.18±0.0				
	ZRef		0.12±0.03	0.12±0.03	0.12±0.03	0.07±0.00
	VRef		0.18±0.01	0.16±0.02	0.18±0.01	0.07±0.03
Microwave		0.18±0.0				
	ZRef		0.15±0.01	0.12±0.01	0.15±0.02	0.08±0.00
	VRef		0.14±0.00	0.14±0.00	0.14±0.00	0.07±0.03

[a]Means are average of 2 values.

Table 17. Lipoxygenase (LOX) activity means and standard deviations for each point related to Figure 11. LOX activity changes in raw (R) rice bran packed in Ziplock® bags (Z) or vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
Raw(Control)		0.18±0.0				
	ZRT		0.21±0.04	0.13±0.04	0.21±0.04	0.04±0.02
	ZRef		0.12±0.03	0.12±0.03	0.12±0.03	0.07±0.00
	VRT		0.20±0.03	0.21±.05	0.20±0.04	0.03±0.00
	VRef		0.18±0.01	0.16±0.02	0.18±0.01	0.07±0.03

[a]Means are average of 2 values.

Table 18. Free fatty acid means and standard deviations for each point related to Figure 12. Free fatty acid changes of raw (R) rice bran packed in Ziplock® bags (Z) or vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
		Free Fatty Acid (%)				
Raw(Control)		2.53±0.2				
	ZRT		34.38±0.9	38.61±3.6	41.99±0.0	48.01±0.1
	ZRef		8.85±0.7	13.42±0.7	15.44±0.1	19.45±1.8
	VRT		38.79±0.2	46.87±1.25	44.89±0.3	54.29±0.6
	VRef		9.27±0.1	17.39±0.8	19.02±0.2	25.37±0.3

[a]Means are average of 2 values.

Table 19. Lipoxygenase (LOX) activity means and standard deviations for each point related to Figure 13. LOX activity changes in microwave (M) heat stabilized rice bran packed in Ziplock® bags (Z) or vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
Microwave		0.18±0.0				
	ZRT		0.37±0.2	0.23±0.04	0.37±0.07	0.54±0.11
	ZRef		0.15±0.01	0.12±0.01	0.15±0.02	0.08±0.00
	VRT		0.22±0.05	0.20±0.00	0.22±0.06	0.26±0.16
	VRef		0.14±0.00	0.14±0.00	0.14±0.00	0.07±0.03

[a]Means are average of 2 values.

Table 20. Free fatty acid means and standard deviations for each point related to Figure 14. Free fatty acid changes of microwave (M) heat stabilized rice bran packed in Ziplock® bags (Z) or vacuum packed (V) stored at room temperature (RT) and in the refrigerator (Ref) during 16 weeks of storage[a].

		Storage Time				
		0 week	4 weeks	8 weeks	12 weeks	16 weeks
Microwave		2.75±0.1				
	ZRT		5.15±0.4	7.610.5	8.10±0.0	10.92±0.6
	ZRef		2.65±0.2	3.85±0.7	3.11±0.2	3.58±0.1
	VRT		5.88±0.1	7.64±0.5	7.52±0.3	11.62±0.0
	VRef		2.89±0.1	4.42±0.1	3.79±0.1	3.74±0.2

[a]Means are average of 2 values.

Table 21. Type III sum of squares (SS's) and associated *p*-values for linoleic acid. The design of study was 2x2x2x4 factor factorial**.

Source	Degree of Freedom	Type III Sum Square	*P*-value
FactorA	1	0.95550625	0.3099
FactorB	1	2.86455625	0.0835
FactorA*FatorB	1	1.02515625	0.2932
FactorC	1	8.52640000	0.0042
FactorA*FactorC	1	0.29702500	0.5691
FactorB*FactorC	1	0.07840000	0.7695
FactorA*FactorB*FactorC	1	0.70560000	0.3819
week	3	56.47685625	0.0001
FactorA*week	3	1.77220625	0.5838
FactorB*week	3	5.83310625	0.1113
FactorA*Factor B*week	3	4.28525625	0.2107
FactorC*week	3	16.48381250	0.0021
FactorA*FactorC*week	3	2.41423750	0.4536
FactorB*FcatorC*week	3	12.14686250	0.0095
FactorA*FactorB*FactorC*week	3	3.80836250	0.2566

The means (average of 2 values) were significantly different at $p < 0.05$ except for interaction of factors A, B, C, and week.

** Factor A = treatment (raw and microwave heat stabilized).
Factor B = packaging methods (Ziplock® bags and vacuum packing).
Factor C = storage temperature (25°C and 4-5°C).
Week = storage period of 4, 8, 12, and 16 weeks.

Table 22. Student-Newman-Keuls (SNK) pairwise comparison test for linoleic acid in rice bran at week 0 and week 16[a].

			Linoleic acid (%)	SNK Grouping
Raw	0 week		32.4	A
	16 weeks	ZRT	32.4	A
		VRT	30.5	A
		ZRef	33.4	A
		VRef	30.2	A
Microwave	0 week		32.6	A
	16 weeks	ZRT	29.9	A
		VRT	30.3	A
		ZRef	34.9	A
		VRef	30.4	A

[a]Means (average of 2 values) with the same letter are not significantly different ($p < 0.05$).
Z = Ziplock® bag, V = vacuum pack, RT = room temperature (25°C), Ref = refrigerator (4-5°C)

Table 23. Student-Newman-Keuls (SNK) pairwise comparison test for moisture content in rice bran at week 0 and week 16[a].

			Moisture (%)	SNK Grouping
Raw	0 week		7.5	B
	16 weeks	ZRT	7.0	BC
		VRT	9.3	A
		ZRef	6.5	C
		VRef	8.5	A
Microwave	0 week		8.4	A
	16 weeks	ZRT	6.4	C
		VRT	6.3	C
		ZRef	6.3	C
		VRef	7.6	B

[a]Means (average of 2 values) with the same letter are not significantly different ($p < 0.05$).
Z = Ziplock® bag, V = vacuum pack, RT = room temperature (25°C), Ref = refrigerator (4-5°C)

VITA

Fatemeh Malekian Ramezanzadeh, the first daughter of Davood Malekian and Zahra Olphat, was born on January 12, 1951, in Tehran, Iran. She graduated as valedictorian from Farideh Salimi High School in May, 1969, and attended Tehran University in August of 1969. As an honor student, she received the bachelor of Science degree in Biology in May, 1973. She married Ghafoor Ramezanzadeh in October of 1969. She is married and has four daughters, Elizeh, Elahe, Julie, and Crystal, and a granddaughter, Sara.

Fatemeh Malekian Ramezanzadeh was accepted into the Graduate School of Louisiana State University in August of 1990. She received her master of science degree in Food Science in May, 1992 and started working as a research associate to establish a Food Analysis Laboratory at Pennington Biomedical Research Center in August of 1992. She is a member of the Institute of Food Technology (IFT), AOAC International, Louisiana State University Kiwanis Club, Faculty Club, Campus Club, and Gamma Sigma Delta the honor society of agriculture. She is presently a candidate for the Doctor of Philosophy degree in Food Science.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Fatemeh Malekian Ramezanzadeh

Major Field: Food Science

Title of Dissertation: Prevention of Hydrolytic and Oxidative Rancidity and Nutrient Losses in Rice Bran During Storage

Approved:

Major Professor and Chairman

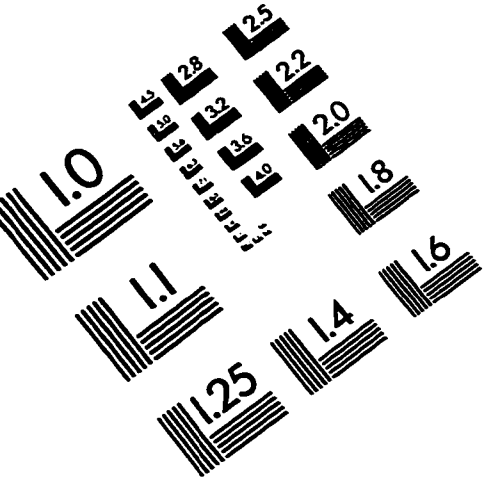
Dean of the Graduate School

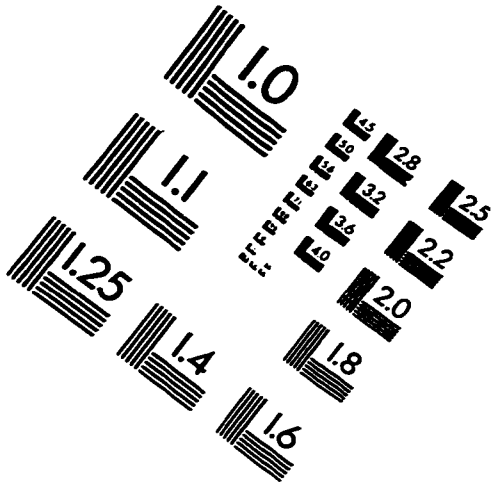
EXAMINING COMMITTEE:

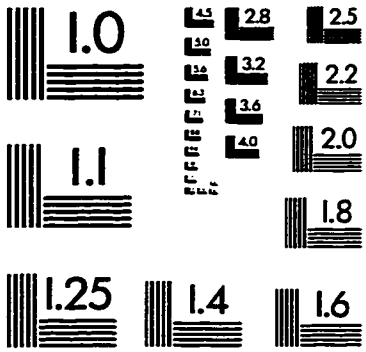
Date of Examination:

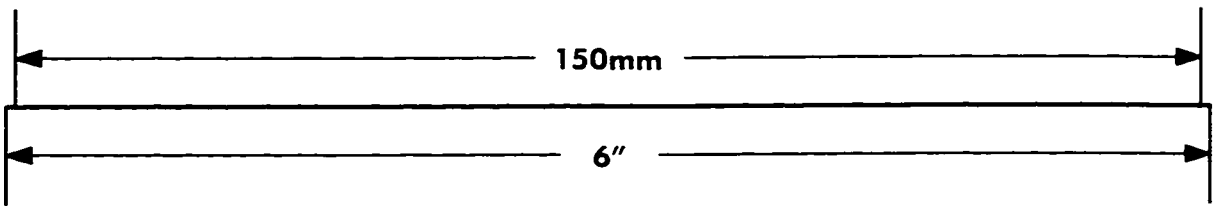
October 30, 1998

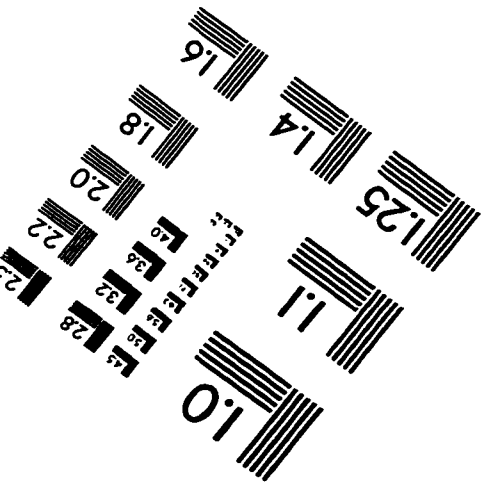
IMAGE EVALUATION
TEST TARGET (QA-3)

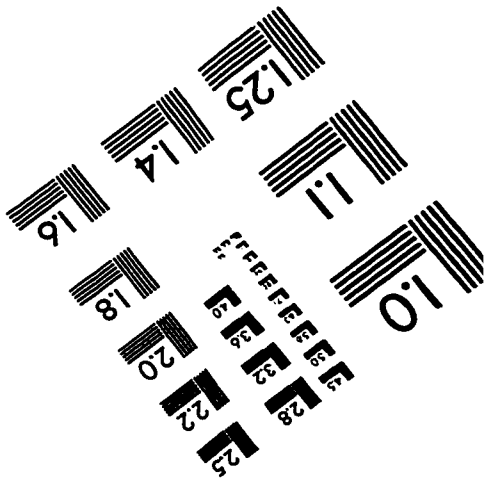












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989